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(71) Applicants *(for all designated States except US)*: **GPC BIOTECH AG** [DE/DE]; Fraunhoferstrasse 20, 82152 Martinsried/München (DE). **MORPHOSYS AG** [DE/DE]; Lena-Christ-Strasse 48, 82152 Martinsried/München (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **NAGY, Zoltan** [DE/US]; One Kendall Square, Bldg. 600, Cambridge, MA 02139 (US). **BRUNNER, Christoph** [DE/DE]; Kreutweg 8, 83673 Bichl (DE). **TESAR, Michael** [DE/DE]; Karolingerstrasse 26, 82362 Weilheim (DE). **THOMASSEN-WOLF, Elisabeth** [DE/DE]; Klingstrasse 12/3, 81369 München (DE).

(74) Agents: **VINCENT, Matthew, P.** et al.; Ropes & Gray, One International Place, Boston, MA 02110-2624 (US).

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(54) **Title:** HUMAN POLYPEPTIDES CAUSING OR LEADING TO THE KILLING OF CELLS INCLUDING LYMPHOID TUMOR CELLS

(57) **Abstract:** The present invention relates to polypeptide compositions which bind to cell surface epitopes and, in multivalent forms, cause or lead to the killing of cells including lymphoid tumor cells, and in the case of monovalent forms, cause immunosuppression or otherwise inhibit activation of lymphocytes. The invention further relates to nucleic acids encoding the polypeptides, methods for the production of the polypeptides, methods for killing cells, methods for immunosuppressing a patient, pharmaceutical, diagnostic and multivalent compositions and kits comprising the polypeptides and uses of the polypeptides.

Human polypeptides causing or leading to the killing of cells including lymphoid tumor cells

Background of the Invention

Every mammalian species, which has been studied to date, carries a cluster of genes coding for the so-called major histocompatibility complex (MHC). This tightly linked cluster of genes code for surface antigens, which play a central role in the development of both humoral and cell-mediated immune responses. In humans the products coded for by the MHC are referred to as Human Leukocyte Antigens or HLA. The MHC-genes are organized into regions encoding three classes of molecules, class I to III.

Class I MHC molecules are 45 kD transmembrane glycoproteins, noncovalently associated with another glycoprotein, the 12 kD beta-2 microglobulin (Brown et al., 1993). The latter is not inserted into the cell membrane, and is encoded outside the MHC. Human class I molecules are of three different isotypes, termed HLA-A, -B, and -C, encoded in separate loci. The tissue expression of class I molecules is ubiquitous and codominant. MHC class I molecules present peptide antigens necessary for the activation of cytotoxic T-cells.

Class II MHC molecules are noncovalently associated heterodimers of two transmembrane glycoproteins, the 35 kD α chain and the 28 kD β chain (Brown et al., 1993). In humans, class II molecules occur as three different isotypes, termed human leukocyte antigen DR (HLA-DR), HLA-DP and HLA-DQ. Polymorphism in DR is restricted to the β chain, whereas both chains are polymorphic in the DP and DQ isotypes. Class II molecules are expressed codominantly, but in contrast to class I, exhibit a restricted tissue distribution: they are present only on the surface of cells of the immune system, for example dendritic cells, macrophages, B lymphocytes, and activated T lymphocytes. They are also expressed on human adrenocortical cells in the zona reticularis of normal adrenal glands and on granulosa-lutein cells in corpora lutea of normal ovaries (Kahoury et al., 1990). Their major biological role is to bind antigenic peptides and present them on the surface of antigen presenting cells (APC) for recognition by CD4 helper T (Th) lymphocytes (Babbitt et al., 1985). MHC class II

molecules can also be expressed on the surface of non-immune system cells, for example, cells that express MHC class II molecules during a pathological inflammatory response. These cells may include synovial cells, endothelial cells, thyroid stromal cells and glial cells.

Class III MHC molecules are also associated with immune responses, but encode somewhat different products. These include a number of soluble serum proteins, enzymes and proteins like tumor necrosis factor or steroid 21-hydroxylase enzymes. In humans, class III molecules occur as three different isotypes, termed Ca, C2 and Bf (Kuby, 1994).

Since Th cell activation is a crucial event of the initiation of virtually all immune responses and is mediated through class II molecules, class II MHC offers itself as a target for immunomodulation (Baxevanis et al., 1980; Rosenbaum et al., 1981; Adorini et al., 1988). Besides peptide presentation, class II molecules can transduce various signals that influence the physiology of APC. Such signals arise by the interaction of multiple class II molecules with an antibody or with the antigen receptor of Th cells (Vidovic et al., 1995a; Vidovic et al., 1995b), and can induce B cell activation and immunoglobulin secretion (Cambier et al., 1991; Palacios et al., 1983), cytokine production by monocytes (Palacios, 1985) as well as the up-regulation of co-stimulatory (Nabavi et al., 1992) and cell adhesion molecules (Mourad et al., 1990).

There is also a set of observations suggesting that class II ligation, under certain conditions, can lead to cell growth arrest or be cytotoxic. Ligation under these conditions is the interaction of a polypeptide with a class II MHC molecule. There is substantial contradiction about the latter effects and their possible mechanisms. Certain authors claim that formation of a complex of class II molecules on B cells leads to growth inhibition (Vaickus et al., 1989; Kabelitz et al., 1989), whereas according to others class II complex formation results in cell death (Vidovic et al., 1995a; Newell et al., 1993; Truman et al., 1994; Truman et al., 1997; Drenou et al., 1999). In certain experimental systems, the phenomenon was observed with resting B cells only (Newell et al., 1993), or in other systems with activated B cells only (Vidovic et al., 1995a; Truman et al., 1994).

Based on these observations, anti-class II monoclonal antibodies (mAbs) have been envisaged for a number of years as therapeutic candidates. Indeed, this proposal has been supported by the beneficial effect of mouse-derived anti-class II mAbs in a series of animal disease models (Waldor et al., 1983; Jonker et al., 1988; Stevens et al., 1990; Smith et al., 1994; Vidovic & Torral, 1998; Vidovic & Laus, 2000).

Despite these early supporting data, to date no anti-MHC class II mAb of human composition has been described that displays the desired cytotoxic and other biological properties which may include affinity, efficiency of killing and selectivity. Indeed, despite the relative ease by which mouse-derived mAbs may be derived, work using mouse-derived mAbs has demonstrated the difficulty of obtaining an antibody with the desired biological properties. For example, significant and not fully understood differences were observed in the T cell inhibitory capacity of different murine anti-class II mAbs (Naquet et al., 1983). Furthermore, the application of certain mouse-derived mAbs *in vivo* was associated with unexpected side effects, sometimes resulting in death of laboratory primates (Billing et al., 1983; Jonker et al., 1991).

It is generally accepted that mouse-derived mAbs (including chimeric and so-called 'humanized' mAbs) carry an increased risk of generating an adverse immune response (Human anti-murine antibody – HAMA) in patients compared to treatment with a human mAb (for example, Vose et al, 2000; Kashmiri et al., 2001). This risk is potentially increased when treating chronic diseases such as rheumatoid arthritis or multiple sclerosis with any mouse-derived mAb or where regular treatment may be required, for example in the treatment of certain cancers; prolonged exposure of the human immune system to a non-human molecule often leads to the development of an adverse immune reaction. Furthermore, it has proven very difficult to obtain mouse-derived antibodies with the desired specificity or affinity to the desired antigen (Pichla et al. 1997). Such observation may significantly reduce the overall therapeutic effect or advantage provided by mouse-derived mAbs. Examples of disadvantages for mouse-derived mAbs may include the following. First, mouse-derived mAbs may be limited in the medical conditions or length of treatment for a condition for which they are appropriate. Second, the dose rate for mouse-derived mAbs may need to be relatively high in order to compensate for a relatively low affinity or therapeutic effect,

hence making the dose not only more severe but potentially more immunogenic and perhaps dangerous. Third, such restrictions in suitable treatment regimes and high-dose rates requiring high production amounts may significantly add to the cost of treatment and could mean that such a mouse-derived mAb be uneconomical to develop as a commercial therapeutic. Finally, even if a mouse mAb could be identified that displayed the desired specificity or affinity, often these desired features are detrimentally affected during the 'humanization' or 'chimerization' procedures necessary to reduce immunogenic potential (Slavin-Chiorini et al., 1997). Once a mouse-derived mAb has been 'humanized' or chimerized, then it is very difficult to optimize its specificity or affinity.

The art has sought over a number of years for anti-MHC class II mAbs of human composition that show biological properties suitable for use in a pharmaceutical composition for the treatment of humans. Workers in the field have practiced the process steps of first identifying a mouse-derived mAb, and then modifying the structure of this mAb with the aim of improving immunotolerance of this non-human molecule for human patients (for further details, see Jones et al., 1986; Riechmann et al., 1988; Presta, 1992). This modification is typically made using so-called 'humanization' procedures or by fabricating a human-mouse chimeric mAb. Other workers have attempted to identify human antibodies that bind to human antigens having desired properties within natural repertoires of human antibody diversity. For example, by exploring the foetal-tolerance mechanism in pregnant women (Bonagura et al., 1987) or by panning libraries of natural diversities of antibodies (Stausbøl-Grøn et al., 1996; Winter et al., 1994). However, to date no anti-MHC class II mAb of human composition has been described that displays the desired biological properties of cytotoxicity, selectivity, specificity, low immunogenicity and affinity.

For therapeutic purposes a polypeptide reacting with many or at least most of the common allelic forms of a human class II MHC molecule would be desirable – e.g., to enable its use in diverse patient populations. Moreover, the candidate polypeptide should be cytotoxic to a wide range of lymphoid tumors, and preferably is cytotoxic by way of a mechanism common to such a range of tumor cells. To allow for a wide range of possible applications, the polypeptide desired should mediate its cytotoxic effect without the dependence on further components of the immune system. For

therapeutic purposes most patients receive for the treatment of e.g. cancer standard chemo- or radiotherapy. Most of these treatments leave the patient immunocompromised. Any additional treatment that relies on an intact immune system is therefore likely to fail. The underlying problem is further demonstrated in humans who suffer from a disease that destroys the immune system, e.g. HIV. Opportunistic infections and malignant transformations are able to escape the immune-surveillance and cause further complications.

Summary of the Invention

One aspect of the present invention relates to a composition including a polypeptide comprising at least one antibody-based antigen-binding domain of human composition with binding specificity for an antigen expressed on the surface of a human cell, wherein treating cells expressing the antigen with a multivalent polypeptide having two or more of said antigen binding domains causes or leads to killing of the cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for killing. In certain in preferred embodiments the antigen is an MHC antigen, preferably an MHC class II antigen, such as DR/DP/DQ or DR. For instance, in certain preferred embodiments, the subject compositions include a polypeptide comprising at least one antibody-based antigen-binding domain which binds to human HLA DR with a K_d of 1µM, 100nM, 10nM or even 1nM or less.

Another aspect of the present invention provides a composition including a multivalent polypeptide comprising a plurality of antibody-based antigen-binding domains of human composition with binding specificity for human HLA DR. Treating cells expressing HLA DR with the multivalent polypetide causes or leads to killing of the cell in a manner where neither cytotoxic entities nor immunological mechanisms are needed for killing. In certain preferred embodiments, the said antigen-binding domains individually bind to the human HLA DR with a K_d of 1µM, 100nM, 10nM or even 1nM or less. In certain preferred embodiments, the multivalent polypeptide has an EC_{50} of 100 nM. 10nM or even 1nM or less for killing activated lymphoid cells, transformed cells and/or lymphoid tumor cells.

Still another aspect of the present invention provides a composition including a polypeptide comprising at least one antibody-based antigen-binding domain that binds to human HLA DR with a K_d of 1µM, 100nM, 10nM or even 1nM or less, the antigen-binding

domain being isolated by a method which includes isolation of human VL and VH domains from a recombinant antibody library by ability to bind to at least one epitope of human HLA DR. Treating a cell expressing HLA DR with a multivalent polypeptide having two or more of the antigen binding domains causes or leads to killing of the cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for killing. In certain embodiments, the method for isolating the antigen-binding domain includes the further steps of:

a. generating a library of variants of at least one of the CDR1, CDR2 and CDR3 sequences of one or both of the VL and VH domains, and
b. isolation of VL and VH domains from the library of variants by ability to bind to human HLA DR with a K_d of 1μM or less.

In certain preferred embodiments, the composition of the present invention can be characterized as including multivalent polypeptides having an EC_{50} for killing transformed cells at least 5-fold lower than the EC_{50} for killing normal cells, and even more preferably at least 10-fold, 100-fold and even 1000-fold less than for killing normal cells.

In certain preferred embodiments, the composition of the present invention are characterized as including multivalent polypeptides having an EC_{50} for killing activated cells at least 5-fold lower than the EC_{50} for killing unactivated cells, and even more preferably at least 10-folded, 100-fold and even 1000-fold less than for killing unactivated cells.

In certain preferred embodiments, the composition of the present invention are characterized as including multivalent polypeptides having an EC_{50} of 50nM or less for killing transformed cells, and even more preferably an EC_{50} of less than 10nM, 1nM and even 0.1nM. In certain embodiments, the subject multivalent polypeptides have an EC_{50} for killing killing activated lymphoid cells, transformed cells and/or lymphoid tumor cells of 100nM, 10nM or even 1nM or less.

In certain embodiments, the subject compositions including multivalent polypeptides selectively kill activated lymphoid cells. For example, such multivalent forms of the subject compositions can be used to kill activated lymphoid cells are lymphoid tumor cells representing a disease selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia, T cell lymphoma, T cell non-Hodgkin lymphoma,

chronic myeloid leukemia, chronic lymphoid leukemia, and multiple myeloid leukemia. Exemplary activated lymphoid tumor cells which can be killed include Priess, GRANTA-519, KARPAS-422, KARPAS-299, DOHH-2, SR-786, MHH-CALL-4, MN-60, BJAB, RAJI, L-428, HDLM-2, HD-MY-Z, KM-H2, L1236, BONNA-12, HC-1, NALM-1, L-363, EOL-1, LP-1, RPMI-8226, and MHH-PREB-1 cell lines. In certain preferred embodiments, the subject compositions have an EC_{50} of 100nM or less, and preferably less than 10nM or even 1nM, for killing at least one of B cell lymphoma cells and T cell lymphoma cells selected from the list of KARPAS-422, DOHH-2, SR-7, MHH-CALL-4, MN-60, HD-MY-Z, NALM-1 and LP-1. In certain instances, to effect cell killing, the target cells may require further activation or pre-activation, such as by by incubation with Lipopolysaccharide (LPS, 10 μg/ml), Interferon-gamma (IFN-γ, Roche, 40 ng/ml) and/or phyto-hemagglutinin (PHA, 5 μg/ml) to name but a few.

In certain embodiments, the multivalent forms of the subject compositions can be used to kill non-lymphoid cells that express MHC class II molecules.

Certain embodiments, one or more the antigen binding domains of the subject compositions bind to the β-chain of HLA-DR, e.g., the antigen-binding domain binds to the first domain of the β-chain of HLA-DR.

In certain other embodiments, one or more the antigen binding domains of the subject compositions bind to the α-chain of HLA-DR, e.g., the antigen-binding domain binds to the first domain of the α-chain of HLA-DR.

In certain preferred embodiments, the the antigen binding domain(s) of the subject compositions bind to one or more HLA-DR types selected from the group consisting of DR1-0101, DR2-15021, DR3-0301, DR4Dw4-0401, DR4Dw10-0402, DR4Dw14-0404, DR6-1302, DR6-1401, DR8-8031, DR9-9012, DRW53-B4*0101 and DRW52-B3*0101. In preferred embodiments, the the antigen binding domains of the subject compositions provide broad-DR reactivity, that is, the antigen-binding domain(s) of a given composition binds to epitopes on at least 5 different of said HLA-DR types. In certain embodiments, the antigen binding domain(s) of a polypeptide(s) of the subject compositions binds to a plurality of HLA-DR types as to bind to HLA DR expressing cells for at least 60 percent of the human population, more preferably at least 75 percent, and even more preferably 85 percent of the human population.

In certain embodiments, the antigen-binding domains of the subject compositions include a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

In certain embodiments, the antigen-binding domains of the subject compositions include a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3, VL CDR1 And VL CDR3 is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

In a further preferred embodiment, the antigen-binding domain is modified compared to a parental antigen-binding domain of the present invention by addition, deletion and/or substitution of amino acid residues, while maintaining the properties according to the present invention, or improving one or more of said properties, of said parental antigen-binding domain. This may include, but is not limited to, the modification of a nucleic acid sequence encoding a parental antigen-binding domain for cloning purposes, the modification of CDR regions in order to improve or modify antigen-binding affinity and/or specificity, including the exchange of one or more CDR sequences of a parental antigen-binding domain by corresponding CDR sequences from one or more different antigen-binding domains, and the addition of peptide sequences for detection and/or purification purposes. It is well within the scope of one of ordinary skill in the art to identify positions in a given parental antigen-binding domain where an addition, deletion and/or substitution should occur, to design and pursue the approach to achieve said addition, deletion and/or substitution, and to test or assay whether the modified antigen-binding domain has maintained the properties of, or exhibits one or more improved properties compared to, the parental antigen-binding domain. Furthermore, one of ordinary skill would be able to design approaches where collections or libraries of modified antigen-binding domains are designed, constructed and screened to identify one or more modified antigen-binding domain which have maintained the properties, or exhibit one or

more improved properties compared to the parental antigen-binding domain. In one example, the first amino acid residue of a HuCAL VH domain comprised in any antigen-binding domain or the present invention, which is either E or Q depending on the expression construct, may be exchanged by Q or E, respectively. Preferred regions to optimize an antigen-binding domain by designing, constructing and screening collections or libraries of modified antigen-binding domains according to the present invention comprise the CDR regions, and most preferably CDR3 of VH and VL, CDR1 of VL and CDR2 of VH domains.

In certain embodiments, the antigen-binding domains includes a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

wherein each n independently represents any amino acid residue; and/or wherein the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

wherein each n independently represents any amino acid residue. For instance, the VH CDR3 sequence can be SPRYGAFDY and/or the VL CDR3 sequence can be QSYDLIRH or QSYDMNVH.

In certain embodiments, the antigen-binding domains of the subject antigen-binding domain competes for antigen binding with an antibody including a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

each n independently represents any amino acid residue; and/or the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

each n independently represents any amino acid residue. For instance, the VH CDR3 sequence can be SPRYGAFDY and/or the VL CDR3 sequence can be QSYDLIRH or QSYDMNVH.

In certain preferred embodiments, the antigen-binding domain includes a VL CDR1 sequence represented in the general formula

SGSnnNIGnNYVn

wherein each n independently represents any amino acid residue. For instance, the CDR1 sequence is SGSESNIGNNYVQ.

In preferred embodiments, the mechanism of killing by multivalent forms of the subject compositions involves an innate pre-programmed process of said cell. For instance, the killing is non-apoptotic. Killing by the subject compositions can be dependent on the action of non-caspase proteases, and/or killing which cannot be inhibited by zVAD-fmk or zDEVD-fmk.

In certain preferred embodiments, the antibody-based antigen-binding domain is part of a multivalent polypeptide including at least a $F(ab')_2$ antibody fragment or a mini-antibody fragment.

In certain preferred embodiments, the antibody-based antigen-binding domain is part of a multivalent polypeptide comprising at least two monovalent antibody fragments selected from Fv, scFv, dsFv and Fab fragments, and further comprises a cross-linking moiety or moieties.

In certain preferred embodiments, the antibody-based antigen-binding domain is part of a multivalent polypeptide comprising at least one full antibody selected from the antibodies of classes IgG1, 2a, 2b, 3, 4, IgA, and IgM.

In certain preferred embodiments, the antibody-based antigen-binding domain is part of a multivalent polypeptide is formed prior to binding to said cell.

In certain preferred embodiments, the antibody-based antigen-binding domain is part of a multivalent polypeptide is formed after binding to said cell.

In certain preferred embodiments, the antigen binding sites are cross-linked to a polymer.

Another aspect of the present invention provides a nucleic acid comprising a coding sequence for an antigen-binding domain, such as those antigen binding domains described above, or a multivalent polypeptide thereof. For example, in certain embodiments, the nucleic acid includes a coding sequence for a polypeptide comprising at least one antibody-based antigen-binding domain of human

composition with binding specificity for an antigen expressed on the surface of a human cell, wherein treating cells expressing the antigen with a multivalent form of the polypeptide causes or leads to killing of said cell in a manner where neither cytotoxic entities nor immunological mechanisms are needed for killing. In certain embodiments, the nucleic acid includes a coding sequence for a polypeptide comprising at least one antibody-based antigen-binding domain which binds to at least one epitope of human HLA DR with a K_d of 1μM, 100nM, 10nM or even 1nM or less.

In certain embodiments, the nucleic acid includes a coding sequence for a polypeptide comprising a plurality of antibody-based antigen-binding domains of human composition with binding specificity for human HLA DR, wherein treating a cell expressing HLA DR with the multivalent polypeptide causes or leads to killing of the cell in a manner where neither cytotoxic entities nor immunological mechanisms are needed for killing. In preferred embodiments, the antigen-binding domains individually bind to epitopes on the human HLA DR with a K_d of 1μM, 100nM, 10nM or even 1nM or less.

In certain embodiments, the nucleic acid includes a coding sequence for a multivalent polypeptide comprising a plurality of antibody-based antigen-binding domains of human composition with binding specificity for human HLA DR, wherein treating a cell expressing HLA DR with said multivalent polypeptide causes or leads to killing of said cell in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said cell killing. Preferably, the multivalent polypeptide has an EC_{50} for killing killing activated lymphoid cells, transformed cells and/or lymphoid tumor cells of 100nM, 10nM or even 1nM or less.

Another aspect of the invention provides a vector comprising the coding sequence of any one of the subject nucleic acids, e.g., as described above, and a transcriptional regulatory sequence operably linked thereto.

Still another aspect of the present invention provides a host cell harboring at least one subject nucleic acids or the subject vector. Another aspect of the present invention provides a method for the production of a multivalent composition that causes or leads to killing of cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed to cause or lead to said killing comprising culturing the host cells under conditions wherein the nucleic acid is expressed either as a polypeptide

comprising a plurality of antigen binding domains or as a polypeptide comprising at least one antigen binding domains which is subsequently treated to form a multivalent composition.

Another aspect of the present invention provides forms of the subject polypeptide or nucleic acid compositions, formulated in a pharmaceutically acceptable carrier and/or diluent. The present invention specifically contemplates the use of such compositions for preparing a pharmaceutical preparation for the treatment of animals, especially humans.

Such pharmaceutical compositions can be used for the treatment of conditions involving unwanted cell proliferation, particularly the treatment of a disorder involving transformed cells expressing MHC class II antigens. For instance, the formulations can be used for the treatment of a disorder selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia, T cell lymphoma, T cell non-Hodgkin lymphoma, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloid leukemia and B cell precursor leukemia.

Such pharmaceutical preparations can be used for the treatment of diseases involving unwanted activation of immune cells, such as in the treatment of a disorder selected from *rheumatoid arthritis, juvenile arthritis, multiple sclerosis*, Grave's disease, insulin-dependent diabetes, narcolepsy, psoriasis, systemic lupus erythematosus, ankylosing spondylitis, transplant rejection, graft vs. host disease, Hashimoto's disease, myasthenia gravis, pemphigus vulgaris, glomerulonephritis, thyroiditis, pancreatitis, insulitis, primary biliary cirrhosis, irritable bowel disease and Sjogren syndrome.

Another aspect of the present invention provides a diagnostic composition including the polypeptide or nucleic acid compositions of the present invention. In certain embodiments, the diagnostic composition includes a polypeptide composition and a cross-linking moiety or moieties.

Still another aspect of the present invention provides a method for killing a cell expressing an antigen on the surface of said cell comprising the step of contacting the cell with a multivalent polypeptide composition of the subject invention.

Another aspect of the invention provides a method to identify patients that can be treated with a multivalent polypeptide composition, formulated in a pharmaceutically acceptable carrier and/or diluent comprising the steps of

a. Isolating cells from a patient;
b. Contacting said cells with the composition; and
c. Measuring the degree of killing or immunosuppression of said cells.

The present invention also provides a kit to identify patients that can be treated with a multivalent polypeptide composition of the present invention, formulated in a pharmaceutically acceptable carrier and/or diluent comprising

a. a multivalent polypeptide composition; and
b. Means to measure the degree of killing or immunosuppression of said cells.

In certain embodiments, the kit includes a multivalent polypeptide composition, and a cross-linking moiety. In other embodiments, the kit includes

a. a multivalent polypeptide composition, and
b. a detectable moiety or moieties, and
c. reagents and/or solutions to effect and/or detect binding of (i) to an antigen.

Another aspect of the present invention provides a cytotoxic composition comprising a multivalent polypeptide composition operably linked to a cytotoxic agent.

Stil another aspect of the invention provides an immunogenic composition comprising a multivalent polypeptide composition operablly linked to an immunogenic agent.

Another aspect of the present invention provides a method to kill a cell comprising contacting the cell with a multivalent polypeptide composition operablly linked a cytotoxic or immunogenic agent.

Another aspect of the invention provides a method for treating a human to reduce the severity of disorder involving unwanted proliferation/activation of cells expressing the human β-chain of HLA DR, comprising administering to the patient a a multivalent polypeptidepolypeptide of the present invention. In certain embodiments, the disorder

involves unwanted proliferation/activation of lymphoid cells, e.g., selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia, T cell lymphoma, T cell non-Hodgkin lymphoma, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloid leukemia and B cell precursor leukemia.

Another aspect of the invention provides a use of a multivalent polypeptide composition operably linked a cytotoxic or immunogenic agent for preparing a pharmaceutical preparation for the treatment of animals

According to a preferred embodiment, the polypeptide is directed to a lymphoid cell or a non-lymphoid cell that expresses MHC class II molecules. The latter type of cells occur for example at pathological sites of inflammation and/or autoimmune diseases, e.g. synovial cells, endothelial cells, thyroid stromal cells and glial cells, or it may also comprise genetically altered cells capable of expressing MHC class II molecules.

Preferably, the polypeptide is directed to lymphoid tumor cells. More preferred are lymphoid tumor cells that represent a disease selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia and B cell precursor leukemia. Most preferred are lymphoid tumor cells from a cell line taken from the list of GRANTA-519, PRIESS, KARPAS-422, DOHH-2, MHH-CALL-4, MN-60, BJAB, L-428, BONNA-12, EOL-1, MHH-PREB-1 and MHH-CALL-2 cell lines.

In certain embodiments, the polypeptide binds to at least one epitope in the alpha-chain of an HLA-DR molecule. In such embodiments, the polypeptide preferably binds to at least one epitope in the first domain of the alpha-chain of HLA-DR, the first domain being the N-terminal domain of the chain. For instance, the polypeptide can be selected to bind to at least one epitope within the alpha-helix ranging from Glu^{55} to Tyr^{79} of the alpha-chain of HLA-DR.

In other embodiments, the polypeptide binds to at least one epitope in the beta-chain of an HLA-DR molecule. Preferably, the polypeptide binds to at least one epitope in the first domain of the beta-chain of HLA-DR, the first domain being the N-terminal domain of the chain.

In certain embodiments, the mechanism of killing a target cell induced by the polypeptide involves an innate pre-programmed process of said cell. Preferably, the polypeptide induces a killing mechanism, which is not an apoptotic cell death process.

In a preferred embodiment the polypeptide induces a killing mechanism which is dependent on the action of proteases other than caspases, e.g., is a caspase-independent mechanism.

In a further embodiment the multivalent composition comprises at least one full antibody which is selected from classes IgG1, 2a, 2b, 3, 4, IgA, and IgM.

In a further embodiment the multivalent composition comprises at least one of a $F(ab')_2$ antibody fragment or mini-antibody fragment.

In a preferred embodiment the multivalent composition comprises at least two monovalent antibody fragments selected from Fv, scFv, dsFv and Fab fragments, and further comprises a cross-linking moiety or moieties.

The present invention also provides a composition including a polypeptide comprising at least one antibody-based antigen-binding domain with a binding specificity for human HLA DR wherein binding of said polypeptide to said epitope causes or leads to suppression of the immune response and wherein said antigen-binding domain includes a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

Another immunosuppressive composition of the present invention includes a polypeptide comprising at least one antibody-based antigen-binding domain with a binding specificity for a human MHC class II antigen with a K_d of 1μM, 100nM, 10nM or even 1nM or less, wherein treating cells expressing MHC class II antigen with the polypeptide causes or

leads to suppression of the immune response, e.g., preferably with an IC50 of 1μM, 100nM, 10nM or even 1nM or less.

Another immunosuppressive composition of the present invention includes a polypeptide comprising at least one antibody-based antigen-binding domain of human composition with a binding specificity for a human MHC class II antigen with a K_d of 1μM , 100nM, 10nM or even 1nM or less, the antigen-binding domain being isolated by a method which includes isolation of human VL and VH domains from a recombinant antibody display library by ability to bind to human MHC class II antigen, wherein treating cells that express MHC class II with said polypeptide causes or leads to suppression of the immune response.

The subject immunosuppressive compositions can be generated using the antigen-binding domain isolated by the further steps of:

a. generating a library of mutations at least one of the CDR1, CDR2 and CDR3 domains of one or both of the VL and VH domains, and

b. isolation of VL and VH domains from the library of variants by ability to bind to human MHC class II antigen with a K_d of 1μM or less.

In preferred embodiments, the antigen binding domains of the immunosuppressive composition binds to HLA-DR, and preferably to the β-chain of HLA-DR, and even more preferably to the first domain of the β-chain of HLA-DR.

In certain preferred embodiments, the immunosuppressive composition have an IC_{50} for suppressing the immune response of 1 μM, 100nM, 10nM or even 1nM or less.

In certain preferred embodiments, the immunosuppressive composition have an IC_{50} for inhibiting of IL-2 secretion of 1 μM, 100nM, 10nM or even 1nM or less.

In certain preferred embodiments, the immunosuppressive composition have an IC_{50} for inhibiting of T cell proliferation of 1 μM, 100nM, 10nM or even 1nM or less.

In certain preferred embodiments, the immunosuppressive composition have antigen-binding domain that bind to an epitope of one or more HLA-DR types selected from the group consisting of DR1-0101, DR2-15021, DR3-0301, DR4Dw4-0401, DR4Dw10-0402,

DR4Dw14-0404, DR6-1302, DR6-1401, DR8-8031, DR9-9012, DRw53-B4*0101 and DRw52-B3*0101, and in preferred embodiments, the antigen-binding domain binds to at least 5 different of said HLA-DR types (e.g., are pan-DR)

In certain embodiments, the immunosuppressive composition have antigen-binding domain includes a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

In certain embodiments, the immunosuppressive composition have antigen-binding domain includes a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

wherein each n independently represents any amino acid residue; and

wherein the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

wherein each n independently represents any amino acid residue.

For instance, the VH CDR3 sequence is SPRYGAFDY and/or the VL CDR3 sequence is QSYDLIRH or QSYDMNVH.

In certain embodiments, the immunosuppressive composition the antigen-binding domain competes with antigen binding by an antibody having a VH CDR3 sequence represented by the general formula

nnnnRGnFDn

wherein each n independently represents any amino acid residue; and

a VL CDR3 sequence represented by the general formula

QSYDnnnn

wherein each n independently represents any amino acid residue.

In certain embodiments, the immunosuppressive composition the antigen-binding domain includes a VL CDR1 sequence represented in the general formula

SGSnnNIGnNYVn

wherein each n independently represents any amino acid residue. For example, the CDR1 sequence is SGSESNIGNNYVQ.

In certain embodiments, the subject immunosuppressive compositions suppress the immune response by one or more of (a) down-regulation of expression of the antigen to which the polypeptide binds; or (b) inhibiting of the interaction between said cell and other cells, wherein said interaction would normally lead to an immune response.

Another aspect of the present invention provides nucleic acids which including a coding sequence for an immunosuppressive polypeptide of the present invention. In certain embodiments, the nucleic acid can be provided as part of a vector, e.g., including the coding sequence and a transcriptional regulatory sequence operably linked thereto. The nucleic acid and vectors of the present invention can be provided as part of a host cell, e.g., which can be used to to produce an immunosuppressive composition.

Another aspect of the present invention provides a method for suppressing activation and/or proliferation of a lymphocyte, comprising contacting the cell with an immunosuppressive polypeptide of the present invention.

The present invention also provides a pharmaceutical preparation comprising the a polypeptide including an antibody-based antigen-binding domain with a binding specificity for a human MHC class II antigen with a K_d of 1µM or less, e.g., in an amount sufficient to suppress an immune response in an animal, inhibit IL-2 secretion in an animal, and/or inhibit T cell proliferation in an animal.

Another aspect of the present invention relates to the use of a polypeptide including an antibody-based antigen-binding domain with a binding specificity for a human MHC class II antigen with a K_d of 1µM or less, for the preparation of a pharmaceutical composition for the treatment of animals, such as where said animals are human.

The subject immunosuppressive pharmaceutical preparations can be used for suppressing IL-2 secretion by a cell of the immune system. For example, these preparations can be administered to the patient in an effective amount to reduce the level of immunological responsiveness in the patient.

Still another aspect of the present invention provides a method for suppressing IL-2 secretion by a lymphocyte, comprising contacting the cell with an immunosuppressive polypeptide of the present invention.

The subject method can be used for immunosuppressing a human, e.g., by administering to the patient an effective amount of an immunosuppressive polypeptide of the present invention to reduce the level of immunological responsiveness.

The invention further relates to a diagnostic composition containing at least one polypeptide and/or nucleic acid according to the invention, optionally together with further reagents, such as buffers, for performing the diagnosis.

In a preferred embodiment the diagnostic composition contains the polypeptide according to the invention cross-linked by at least one moiety. Such moieties can be for example antibodies recognizing an epitope present on the polypeptide such as the FLAG peptide epitope (Hopp et al., 1988; Knappik and Plückthun, 1994) or bifunctional chemical compounds reacting with a nucleophilic amino acid side chain as present in cysteine or lysine (King et al., 1994). Methods for cross-linking polypeptides are well known to the practitioner of ordinary skill in the art.

A diagnostic composition containing at least one nucleic acid and/or variant thereof according to the invention is also contemplated.

Furthermore, the present invention relates to a kit comprising at least one polypeptide according to the present invention, and a cross-linking moiety.

Additionally, the present invention relates to a kit comprising (i) a polypeptide according to the present invention, (ii) a detectable moiety or moieties, and (iii) reagents and/or solutions to effect and/or detect binding of (i) to an antigen.

The present invention further relates to a multivalent composition comprising at least one polypeptide and comprising at least two antigen binding domains.

Still another aspect of the present invention provides a method for conducting a pharmaceutical business comprising:

(i) isolating one or more antigen-binding domains that bind to antigens expressed on the surface of human cells;

(ii) generating a multivalent composition comprising a plurality of said antigen-binding domains, which multivalent composition kills with an EC_{50} of 50nM or less transformed or activated cells where neither cytotoxic entities nor immunological mechanisms are needed to cause or lead to said killing.;

(iii) conducting therapeutic profiling of the multivalent compositions for efficacy and toxicity in animals;

(iv) preparing a package insert describing the multivalent composition for treatment of proliferative disorders; and

(v) marketing the multivalent composition for treatment of proliferative disorders.

The present invention also provides a method for conducting a life science business comprising:

(i) isolating one or more antigen-binding domains that bind to antigens expressed on the surface of human cells;

(ii) generating a multivalent composition comprising a plurality of said antigen-binding domains, which multivalent composition kills with an EC_{50} of 50nM or less transformed or activated cells where neither cytotoxic entities nor immunological mechanisms are needed to cause or lead to said killing.;

(iii) licensing, jointly developing or selling, to a third party, the rights for selling the multivalent compositions.

In such embodiments, the the antigen-binding domain can be isolated by a method which includes

a. isolation of VL and VH domains of human composition from a recombinant antibody display library by ability to bind to epitopes of HLA DR,

b. generating a library of variants at least one of the CDR1, CDR2 and CDR3 domains of one or both of the VL and VH domains, and

c. isolation of VL and VH domains from the library of variants by ability to epitopes of HLA DR with a K_d of 1μM or less.

Another business method contemplated by the present invention includes:

(i) isolating one or more antigen-binding domains that bind to MHC class II expressed on the surface of human cells with a K_d of 1μM or less;

(ii) generating a composition comprising said antigen-binding domains, which composition is immunosuppressant with an IC_{50} of 100nM or less;

(iii) conducting therapeutic profiling of the multivalent compositions for efficacy and toxicity in animals;

(iv) preparing a package insert describing the use of the composition for immunosuppression therapy; and

(v) marketing the multivalent composition for use as an immunosuppressant.

The present invention also provides a method for conducting a life science business comprising:

(i) isolating one or more antigen-binding domains that bind to MHC class II expressed on the surface of human cells with a K_d of 1µM or less;

(ii) generating a composition comprising said antigen-binding domains, which composition is immunosuppressant with an IC_{50} of 100nM or less;

(iii) licensing, jointly developing or selling, to a third party, the rights for selling the compositions.

As used herein, the term "peptide" relates to molecules consisting of one or more chains of multiple, i. e. two or more, amino acids linked via peptide bonds.

The term "protein" refers to peptides where at least part of the peptide has or is able to acquire a defined three-dimensional arrangement by forming secondary, tertiary, or quaternary structures within and/or between its peptide chain(s). This definition comprises proteins such as naturally occurring or at least partially artificial proteins, as well as fragments or domains of whole proteins, as long as these fragments or domains are able to acquire a defined three-dimensional arrangement as described above.

The term “polypeptide” is used interchangeably to refer to peptides and/or proteins. Moreover, the terms “polypeptide “ and “protein”, as the context will admit, include multi-chain protein complexes, such as immunoglobulin polypeptides having separate heavy and light chains.

In this context, "polypeptide comprising at least one antibody-based antigen-binding domain" refers to an immunoglobulin (or antibody) or to a fragment thereof. The term

"fragment", with respect to antibody domains and the like, refers to a fragment of an immunoglobulin which retains the antigen-binding moiety of an immunoglobulin. Functional immunoglobulin fragments according to the present invention may be Fv (Skerra and Plückthun, 1988), scFv (Bird et al., 1988; Huston et al., 1988), disulfide-linked Fv (Glockshuber et al., 1992; Brinkmann et al., 1993), Fab, $F(ab')_2$ fragments or other fragments well-known to the practitioner skilled in the art, which comprise the variable domains of an immunoglobulin or functional immunoglobulin fragment.

Examples of polypeptides consisting of one chain are single-chain Fv antibody fragments, and examples for polypeptides consisting of multiple chains are Fab antibody fragments.

The term "antibody" as used herein, unless indicated otherwise, is used broadly to refer to both antibody molecules and a variety of antibody derived molecules. Such antibody derived molecules comprise at least one variable region (either a heavy chain of light chain variable region) and include such fragments as described above, as well as individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, and the like.

The "antigen-binding site" of an immunoglobulin molecule refers to that portion of the molecule that is necessary for binding specifically to an antigen. An antigen binding site preferably binds to an antigen with a Kd of 1μM or less, and more preferably less than 100nM, 10nM or even 1nM in certain instances. Binding specifically to an antigen is intended to include binding to the antigen which significantly higher affinity than binding to any other antigen.

The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus the term "FR" refers to amino acid sequences which are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to

each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs."

For the purposes of this application, "valent" refers to the number of antigen binding sites the subject polypeptide possess. Thus, a bivalent polypeptide refers to a polypeptide with two binding sites. The term "multivalent polypeptide" encompasses bivalent, trivalent, tetravalent, etc. forms of the polypeptide.

As used herein, a "multivalent composition" means a composition comprising a polypeptide having at least two of said antigen-binding domains, e.g.,a multivalent polypeptide. Preferably, said at least two antigen-binding domains are in close proximity so as to mimic the structural arrangement relative to each other of binding sites comprised in a full immunoglobulin molecule. Examples for multivalent compositions are full immunoglobulin molecules (e.g. IgG, IgA or IgM molecules) or multivalent fragments thereof (e.g. $F(ab')_2$). Additionally, multivalent compositions of higher valencies may be formed from two or more multivalent compositions (e.g. two or more full immunoglobulin molecules), e.g. by cross-linking. Multivalent compositions, however, may be formed as well from two or more monovalent immunoglobulin fragments, e.g. by self-association as in mini-antibodies, or by cross-linking.

Accordingly, an "antibody-based antigen-binding domain" refers to polypeptide or polypeptides which form an antigen-binding site retaining at least some of the structural features of an antibody, such as at least one CDR sequence. In certain preferred embodiments, antibody-based antigen-binding domain includes sufficient structure to be considered a variable domain, such as three CDR regions and interspersed framework regions. Antibody-based antigen-binding domain can be formed single polypeptide chains corresponding to VH or VL sequences, or by intermolecular or intramolecular association of VH and VL sequences.

The term "recombinant antibody library" describes a variegated library of antigen binding domains. For instance, the term includes a collection of display packages,

e.g., biological particles, which each have (a) genetic information for expressing at least one antigen binding domain on the surface of the particle, and (b) genetic information for providing the particle with the ability to replicate. For instance, the package can display a fusion protein including an antigen binding domain. The antigen binding domain portion of the fusion protein is presented by the display package in a context which permits the antigen binding domain to bind to a target epitope that is contacted with the display package. The display package will generally be derived from a system that allows the sampling of very large variegated antibody libraries. The display package can be, for example, derived from vegetative bacterial cells, bacterial spores, and bacterial viruses.

In an exemplary embodiment of the present invention, the display package is a phage particle which comprises a peptide fusion coat protein that includes the amino acid sequence of a test antigen binding domains. Thus, a library of replicable phage vectors, especially phagemids (as defined herein), encoding a library of peptide fusion coat proteins is generated and used to transform suitable host cells. Phage particles formed from the chimeric protein can be separated by affinity selection based on the ability of the antigen binding site associated with a particular phage particle to specifically bind a target eptipope. In a preferred embodiment, each individual phage particle of the library includes a copy of the corresponding phagemid encoding the peptide fusion coat protein displayed on the surface of that package. Exemplary phage for generating the present variegated peptide libraries include M13, f1, fd, If1, Ike, Xf, Pf1, Pf3, λ, T4, T7, P2, P4, ϕX-174, MS2 and f2.

The term "generating a library of variants of at least one of the CDR1, CDR2 and CDR3" refers to a process of generating a library of variant antigen binding sites in which the members of the library differ by one or more changes in CDR sequences, e.g., not FR sequences. Such libraries can be generated by random or semi-random mutagenesis of one or more CDR sequences from a selected antigen binding site.

As used herein, an "antibody-based antigen-binding domain of human composition" preferably means a polypeptide comprising at least an antibody VH domain and an antibody VL domain, wherein a homology search in a database of protein sequences comprising immunoglobulin sequences results for both the VH and the VL domain in

an immunoglobulin domain of human origin as hit with the highest degree of sequence identity. Such a homology search may be a BLAST search, e.g. by accessing sequence databases available through the National Center for Biological Information and performing a "BasicBLAST" search using the "blastp" routine. See also Altschul et al. (1990) J Mol Biol 215:403-410. Preferably, such a composition does not result in an adverse immune response thereto when administered to a human recipient. In certain preferred embodiments, the subject antigen-binding domains of human composition include the framework regions of native human immunoglobulins, as may be cloned from activated human B cells, though not necessarily all of the CDRs of a native human antibody.

As used herein, the term “mini-antibody fragment” means a multivalent antibody fragment comprising at least two antigen-binding domains multimerized by self-associating domains fused to each of said domains (Pack, 1994), e.g. dimers comprising two scFv fragments, each fused to a self-associating dimerization domain. Dimerization domains, which are particularly preferred, include those derived from a leucine zipper (Pack and Plückthun, 1992) or helix-turn-helix motif (Pack et al., 1993).

As used herein, “activated cells” means cells of a certain population of interest, which are not resting. Activation might be caused by mitogens (e.g., lipopoysaccharide, phytohemagglutinine) or cytokines (e.g., interferon gamma). Preferably, said activation occurs during tumor transformation (e.g., by Epstein-Barr virus, or “spontaneously”). Preferably, activated cells are characterized by the features of MHC class II molecules expressed on the cell surface and one or more additional features including increased cell size, cell division, DNA replication, expression of CD45 or CD11 and production/secretion of immunoglobulin.

As used herein, “non-activated cells” means cells of a population of interest, which are resting and non-dividing. Said non-activated cells may include resting B cells as purified from healthy human blood. Such cells can, preferably, be characterized by lack or reduced level of MHC class II molecules expressed on the cell surface and lack or reduced level of one or more additional features including increased cell size, cell division, DNA replication, expression of CD45 or CD11 and production/secretion of immunoglobulin.

As used herein, the term “EC50” means the concentration of multivalent forms of the subject compositions which produces 50% of its maximum response or effect, such as cell killing.

"At least 5-fold lower EC50" means that the concentration of a multivalent composition comprising at least one polypeptide of the present invention that is required to kill 50% of activated cells is at least five times less than the concentration of the multivalent composition required to kill non-activated cells. Preferably, the concentration required to kill 50% of non-activated cells cannot be achieved with therapeutically appropriate concentrations of the multivalent composition. Most preferably, the EC50 value is determined in the test described below in the appended examples.

The term “immunosuppress” refers to the prevention or diminution of the immune response, as by irradiation or by administration of antimetabolites, antilymphocyte serum, or specific antibody.

The term “immune response” refers to any response of the immune system, or a cell forming part of the immune system (lymphocytes, granulocytes, macrophages, etc), to an antigenic stimulus, including, without limitation, antibody production, cell-mediated immunity, and immunological tolerance.

As used herein, the term “IC50” with respect immunosuppression, refers to the concentration of the subject compositions which produces 50% of its maximum response or effect, such as inhibition of an immune response, such as may be manifest by inhibition of IL2 secretion, down-regulation of IL2 expression, or reduced rate of cell proliferation.

The phrase “cytotoxic entities”, with reference to a manner of cell killing, refers to mechanisms which are complement-dependent. Likewise, the phrase “immuological mechanism” , with reference to a manner of cell killing, refers to macrophage-dependent and/or neutrophil-dependent killing of cells.

"Lymphoid cells" when used in reference to a cell line or a cell, means that the cell line or cell is derived from the lymphoid lineage. "Lymphoid cells" include cells of the B and the T lymphocyte lineages, and of the macrophage lineage.

Cells, which are "non lymphoid cells and express MHC class II", are cells other than lymphoid cells that express MHC class II molecules, e.g. during a pathological inflammatory response. For example, said cells may include synovial cells, endothelial cells, thyroid stromal cells and glial cells, and it may also comprise genetically altered cells capable of expressing MHC class II molecules.

The terms "apoptosis" and "apoptotic activity" refer to the form of cell death in mammals that is accompanied by one or more characteristic morphological and biochemical features, including nuclear and condensation of cytoplasm, chromatin aggregation, loss of plasma membrane microvilli, partition of cytoplasm and nucleus into membrane bound vesicles (apoptotic bodies) which contain ribosomes, morphologically intact mitochondria and nuclear material, degradation of chromosomal DNA or loss of mitochondrial function. Apoptosis follows a very stringent time course and is executed by caspases, a specific group of proteases. Apoptotic activity can be determined and measured, for instance, by cell viability assays, Annexin V *staining or caspase inhibition assays. Apoptosis can be induced* using a cross-linking antibody such as anti-CD95 as described in Example H.

As used herein, the term "first domain of the α-chain of HLA-DR" means the N-terminal domain of the alpha-chain of the MHC class II DR molecule.

As used herein, the term "first domain of the β-chain of HLA-DR" means the N-terminal domain of the beta-chain of the MHC class II DR molecule.

The term "innate pre-programmed process" refers to a process that, once it is started, follows an autonomous cascade of mechanisms within a cell, which does not require any further auxillary support from the environment of said cell in order to complete the process.

As used herein, the term "HuCAL" refers to a fully synthetic human combinatorial antibody library as described in Knappik et al. (2000).

The term "variable region" as used herein in reference to immunoglobulin molecules has the ordinary meaning given to the term by the person of ordinary skill in the act of immunology. Both antibody heavy chains and antibody light chains may be divided into a "variable region" and a "constant region". The point of division between a variable region and a heavy region may readily be determined by the person of ordinary skill in the art by reference to standard texts describing antibody structure, e.g., Kabat et al "Sequences of Proteins of Immunological Interest: 5th Edition" U.S. Department of Health and Human Services, U.S. Government Printing Office (1991).

As used herein, the term "CDR3" refers to the third complementarity-determining region of the VH and VL domains of antibodies or fragments thereof, wherein the VH CDR3 covers positions 95 to 102 (possible insertions after positions 100 listed as 100a to 100z), and VL CDR3 positions 89 to 96 (possible insertions in Vλ after position 95 listed as 95a to 95c) (see Knappik et al., 2000).

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least 65%, more preferably at least 70%, and even more preferably at least 75% homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, New York. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions is hybridization in 6 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50°-65°C.

A “protein coding sequence” or a sequence which “encodes” a particular polypeptide or peptide, is a nucleic acid sequence which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5’ (amino) terminus and a

translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eukaryotic mRNA, genomic DNA sequences from procaryotic or eukaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

Likewise, "encodes", unless evident from its context, will be meant to include DNA sequences which encode a polypeptide, as the term is typically used, as well as DNA sequences which are transcribed into inhibitory antisense molecules.

As used herein, the term "transfection" means the introduction of a heterologous nucleic acid, e.g., an expression vector, into a recipient cell by nucleic acid-mediated gene transfer. "Transient transfection" refers to cases where exogenous DNA does not integrate into the genome of a transfected cell, e.g., where episomal DNA is transcribed into mRNA and translated into protein. A cell has been "stably transfected" with a nucleic acid construct when the nucleic acid construct is capable of being inherited by daughter cells.

"Expression vector" refers to a replicable DNA construct used to express DNA which encodes the desired protein and which includes a transcriptional unit comprising an assembly of (1) agent(s) having a regulatory role in gene expression, for example, promoters, operators, or enhancers, operatively linked to (2) a DNA sequence encoding a desired protein (such as a polypeptide of the present invention) which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. The choice of promoter and other regulatory elements generally varies according to the intended host cell. In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

In the expression vectors, regulatory elements controlling transcription or translation can be generally derived from mammalian, microbial, viral or insect genes The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated. Vectors derived from viruses, such as retroviruses, adenoviruses, and the like, may be employed.

"Transcriptional regulatory sequence" is a generic term used throughout the specification to refer to DNA sequences, such as initiation signals, enhancers, and promoters and the like which induce or control transcription of protein coding sequences with which they are operably linked. It will be understood that a recombinant gene can be under the control of transcriptional regulatory sequences which are the same or which are different from those sequences which control transcription of the naturally-occurring form of the gene, if any.

"Operably linked" when describing the relationship between two DNA regions simply means that they are functionally related to each other. For example, a promoter or other transcriptional regulatory sequence is operably linked to a coding sequence if it controls the transcription of the coding sequence.

As used herein, the term "fusion protein" is art recognized and refer to a chimeric protein which is at least initially expressed as single chain protein comprised of amino acid sequences derived from two or more different proteins, e.g., the fusion protein is a gene product of a fusion gene.

As used herein, "proliferating" and "proliferation" refer to cells undergoing mitosis.

The "growth rate" of a cell refers to the rate of proliferation of the cell and the state of differentiation of the cell.

The term "cell-proliferative disorder" denotes malignant as well as nonmalignant populations of transformed cells which morphologically often appear to differ from the surrounding tissue.

As used herein, "transformed cells" refers to cells which have spontaneously converted to a state of unrestrained growth, i.e., they have acquired the ability to grow through an indefinite number of divisions in culture. Transformed cells may be characterized by such terms as neoplastic, anaplastic and/or hyperplastic, with respect to their loss of growth control.

As used herein, "immortalized cells" refers to cells which have been altered via chemical and/or recombinant means such that the cells have the ability to grow through an indefinite number of divisions in culture.

As used herein the term "animal" refers to mammals, preferably mammals such as humans. Likewise, a "patient" or "subject" to be treated by the method of the invention can mean either a human or non-human animal.

According to the methods of the invention, the peptide may be administered in a pharmaceutically acceptable composition. In general, pharmaceutically-acceptable carriers for monoclonal antibodies, antibody fragments, and peptides are well-known to those of ordinary skill in the art. As used herein, the term "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. In preferred embodiments, the subject carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not excessively toxic to the hosts of the concentrations of which it is administered. The administration(s) may take place by any suitable technique, including subcutaneous and parenteral administration, preferably parenteral. Examples of parenteral administration include intravenous, intraarterial, intramuscular, and intraperitoneal, with intravenous being preferred.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or

dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds, e.g., the subject polypeptides, in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

For oral administration the polypeptides of the present invention may be incorporated with excipients and used in the form of non-ingestible mouthwashes and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). The active ingredient may also be dispersed in dentifrices, including: gels, pastes, powders and slurries. The active ingredient may be added in a therapeutically effective amount to a paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants.

The compositions of the present invention may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free

amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

Upon formulation, solutions can be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

As used herein, the term "prophylactic or therapeutic" treatment refers to administration to the host of the medical condition. If it is administered prior to exposure to the condition, the treatment is prophylactic (i.e., it protects the host against tumor formation), whereas if administered after initiation of the disease, the treatment is therapeutic (i.e., it combats the existing tumor).

A multivalent composition of at least one polypeptide according to the invention is capable of causing cell death of activated cells, preferably lymphoid tumor cells without requiring any further additional measures such as chemotherapy and with limited immunogenic side effects on the treated patient. Further, the multivalent composition comprising a polypeptide according to the invention has the capability of binding to at least one epitope on the target antigen, however, several epitope binding sites might be combined in one molecule. Preferably, the multivalent composition comprising a polypeptide according to the invention shows at least 5-fold, or more preferably 10-fold higher killing activity against activated cells compared to non-activated cells. This higher activity on activated cells can be expressed as the at least 5-fold lower EC50 value on activated versus non-activated cells or as the higher percentage of killing of activated cells versus non-activated cells when using the same concentration of protein. Under the latter alternative, the multivalent composition comprising a polypeptide according to the invention at a given polypeptide concentration kills at least 50%, preferably at least 80%, of activated cells, whereas the same concentration of a multivalent composition comprising a polypeptide according to the invention under the same incubation conditions kills less than 15%, preferably less than 10% of the non-activated cells. The assay conditions for determining the EC50 value and the percentage killing activity are described below.

Brief Description of the Drawings

Figure 1

a. Specificity of the anti-HLA-DR antibody fragments: Binding of MS-GPC-8-27-7, MS-GPC-8-27-10, MS-GPC-8-6-13, MS-GPC-8-27-41, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-6-27, MS-GPC-8 and MS-GPC-8-6 to HLA-DR protein, negative control proteins (BSA, testosterone-BSA, lysozyme and human apotransferrin), and an empty microtiter plate well (plastic). Specificity was assessed using standard ELISA procedures.

b. Specificity of the anti-HLA-DR antibody fragments MS-GPC-1, 6, 8 & 10 isolated from the HuCAL library to HLA-DR protein, a mouse-human chimeric HLA protein and negative control proteins (lysozyme, transferrin, BSA and human β-globulin).

Specificity was assessed using standard ELISA procedures. A non-related antibody fragment (irr. scFv) was used as control.

Figure 2

Reactivity of the anti-HLA-DR antibody fragments (MS-GPC-1, 6, 8 and 10) and IgG forms of MS-GPC-8, MS-GPC-8-10-57, MS-GPC-8-27-41 & MS-GPC-8-6-17 to various cell lines expressing MHC class II molecules. "+" represents strong reactivity as detected using standard immunofluorescence procedure. "+/-" represents weak reactivity and "-" represents no detected reactivity between an anti-HLA-DR antibody fragment or IgG and a particular cell line.

Figure 3

Viability of tumor cells in the presence of monovalent and cross-linked anti-HLA-DR antibody fragments as assessed by trypan blue staining. Viability of GRANTA-519 cells was assessed after 4 h incubation with anti-HLA-DR antibody fragments (MS-GPC-1, 6, 8 and 10) with and without anti-FLAG M2 mAb as cross-linking agent.

Figure 4

Scatter plots and fitted logistic curves of data from Table 5 showing improved killing efficiency of 50 nM solutions of the IgG form of the human antibody fragments of the invention treated compared to treatment with 200 nM solutions of murine antibodies. Open circles represent data for cell lines treated with the murine antibodies L243 and 8D1 and closed circles for human antibodies MS-GPC-8, MS-GPC-8-27-41, MS-GPC-8-10-57 and MS-GPC-8-6-13. Fitted logistic curves for human (solid) and mouse (dashed) mAb cell killing data show the overall superiority of the treatment with human mAbs at 50 nM compared to the mouse mAbs despite treatment at a final concentration of 200 nM.

Figure 5

Killing of activated versus non-activated cells. MHH-PREB-1 cells are activated with Lipopolysaccharide, Interferon-gamma and phyto-hemagglutin, and subsequently incubated for 4 h with 0.07 to 3300 nM of the IgG forms of the anti-HLA-DR antibody

fragments MS-GPC-8-10-57 and MS-GPC-8-27-41. No loss of viability in the control non-activated MHH-PREB-1 cells is seen.

Figure 6

Killing efficiency of control (no antibody, unreactive murine IgG; light grey), and human (MS-GPC-8, MS-GPC-8-10-57 & MS-GPC-8-27-41; dark grey) IgG forms of anti-HLA-DR antibody fragments against CLL cells isolated from patients. Left panel, box-plot display of viability data from 10 patient resting cell cultures against antibodies after incubation for four (h4) and twenty four hours (h24). Right panel box-plot display of viability data from 6 patient activated cell cultures against antibodies after incubation for four (h4) and twenty four hours (h24).

Figure 7

Concentration dependent cell viability for certain anti-HLA-DR antibody fragments of the invention. Vertical lines indicate the EC50 value estimated by logistic non-linear regression on replica data obtained for each of the antibody fragments. a) Killing curves of cross-linked bivalent anti-HLA-DR antibody F(ab) fragment dimers MS-GPC-10 (circles and solid line), MS-GPC-8 (triangles and dashed line) and MS-GPC-1 (crosses and dotted line). b) Killing curves of cross-linked bivalent anti-HLA-DR antibody (Fab) fragment dimers MS-GPC-8-17 (circles and solid line), and murine IgGs 8D1 (triangles and dashed line) and L243 (crosses and dotted line). c) Killing curves of cross-linked bivalent anti-HLA-DR antibody (Fab) fragment dimers GPC-8-6-2 (crostriangles and dashed line), and murine IgGs 8D1 (circles and solid line) and L243 (crosses and dotted line). d) Killing curves of IgG forms of human anti-HLA-DR antibody fragments MS-GPC-8-10-57 (crosses and dotted line), MS-GPC-8-27-41 (exes and dash-dot line), and murine IgGs 8D1 (circles and solid line) and L243 (triangles and dashed line). All concentrations are given in nM of the bivalent agent (IgG or cross-linked (Fab) dimer).

Figure 8

a. Incubation of Priess cells with the anti-HLA-DR antibody fragment MS-GPC-8, cross-linked using the anti-FLAG M2 mAb, shows more rapid killing than a culture of

Priess cells induced into apoptosis using anti-CD95 mAb. An Annexin V/PI staining technique identifies necrotic cells by Annexin V positive and PI positive staining.

b.Incubation of Priess cells with the anti-HLA-DR antibody fragment MS-GPC-8, cross-linked using the anti-FLAG M2 mAb, shows little evidence of an apoptotic mechanism compared to an apoptotic culture of Priess cells induced using anti-CD95 mAb. An Annexin V/PI staining technique identifies apoptotic cells by Annexin V positive and PI negative staining.

Figure 9

a. Immunosuppressive properties of the IgG forms of the anti-HLA-DR antibody fragments MS-GPC-8-10-57, MS-GPC-8-27-41 & MS-GPC-8-6-13 using an assay to determine inhibition of IL-2 secretion from T-hybridoma cells.

b. Immunosuppressive properties of the monovalent Fab forms of the anti-HLA-DR antibody fragments MS-GPC-8-27-41 & MS-GPC-8-6-19 using an assay to determine inhibition of IL-2 secretion from T-hybridoma cells

Figure 10

Immunosuppressive properties of the IgG forms of the anti-HLA-DR antibody fragments MS-GPC-8-10-57 and MS-GPC-8-27-41 in an assay to determine inhibition of T cell proliferation.

Figure 11

Vector map and sequence of scFv phage display vector pMORPH13_scFv.
The vector pMORPH13_scFv is a phagemid vector comprising a gene encoding a fusion between the C-terminal domain of the gene III protein of filamentous phage and a HuCAL scFv. In Figure 11, a vector comprising a model scFv gene (combination of VH1A and Vλ3 (Knappik et al., 2000) is shown.
The original HuCAL master genes (Knappik et al. (2000): see Fig. 3 therein) have been constructed with their authentic N-termini: VH1A, VH1B, VH2, VH4 and VH6 with Q (=CAG) as the first amino acid. VH3 and VH5 with E (=GAA) as the first amino acid. Vector pMORPH13_scFv comprises the short FLAG peptide sequence (DYKD) fused to the VH chain, and thus all HuCAL VH chains in, and directly derived from,

this vector have E (=GAA) at the first position (e.g. in pMx7_FS vector, see Figure 12).

Figure 12

Vector map and sequence of scFv expression vector pMx7_FS_5D2.

The expression vector pMx7_FS_5D2 leads to the expression of HuCAL scFv fragments (in Figure 12, the vector comprises a gene encoding a "dummy" antibody fragment called "5D2") when VH-CH1 is fused to a combination of a FLAG tag (Hopp et al., 1988; Knappik and Plückthun, 1994) and a STREP tag II (WSHPQFEK) (IBA GmbH, Göttingen, Germany; see: Schmidt and Skerra, 1993; Schmidt and Skerra, 1994; Schmidt et al., 1996; Voss and Skerra, 1997).

Figure 13

Vector map and sequence of Fab expression vector pMx9_Fab_GPC8.

The expression vector pMx9_Fab_GPC8 leads to the expression of HuCAL Fab fragments (in Figure 13, the vector comprises the Fab fragment MS-GPC8) when VH-CH1 is fused to a combination of a FLAG tag (Hopp et al., 1988; Knappik and Plückthun, 1994) and a STREP tag II (WSHPQFEK) (IBA GmbH, Göttingen, Germany; see: Schmidt and Skerra, 1993; Schmidt and Skerra, 1994; Schmidt et al., 1996; Voss and Skerra, 1997).

In pMx9_Fab vectors, the HuCAL Fab fragments cloned from the scFv fragments (see figure caption of Figure 11) do not have the short FLAG peptide sequence (DYKD) fused to the VH chain, and all HuCAL VH chains in, and directly derived from, that vector have Q (=CAG) at the first position

Figure 14

Vector map and sequence of Fab phage display vector pMORPH18_Fab_GPC8.

The derivatives of vector pMORPH18 are phagemid vectors comprising a gene encoding a fusion between the C-terminal domain of the gene III protein of filamentous phage and the VH-CH1 chain of a HuCAL antibody. Additionally, the vector comprises the separately encoded VL-CL chain. In Figure 14, a vector comprising the Fab fragment MS-GPC-8 is shown.

In pMORPH18_Fab vectors, the HuCAL Fab fragments cloned from the scFv fragments (see figure caption of Figure 11) do not have the short FLAG peptide

sequence (DYKD) fused to the VH chain, and all HuCAL VH chains in, and directly derived from, that vector have Q (=CAG) at the first position.

Figure 15

Amino acid sequences of VH and VL domains of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-6, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-27, MS-GPC-8-6-13, MS-GPC-8-10-57, and MS-GPC-8-27-41.

The sequences in Figure 15 show amino acid 1 of VH as constructed in the original HuCAL master genes (Knappik et al. (2000): see Fig. 3 therein). In scFv constructs, as described in this application, amino acid 1 of VH is always E (see figure caption of Figure 11), in Fab constructs as described in this application, amino acid 1 of VH is always Q (see figure caption of Figure 13)

Detailed Description of the Invention

The following examples illustrate the invention.

Examples

All buffers, solutions or procedures without explicit reference can be found in standard textbooks, for example Current Protocols of Immunology (1997 and 1999) or Sambrook et al., 1989. Where not given otherwise, all materials were purchased from Sigma, Deisenhofen, DE, or Merck, Darmstadt, DE, or sources are given in the literature cited. Hybridoma cell lines LB3.1 and L243 were obtained from LGC Reference Materials, Middlesex, UK; data on antibody 8D1 were generously supplied by Dr. Matyas Sandor, University of Michigan, Madison, WI, USA.

1. Preparation of a human antigen

To demonstrate that we could identify cytotoxic antigen-binding domains of human composition, we first prepared a purified form of a human antigen, the human MHC class II DR protein (DRA*0101/DRB1*0401) from PRIESS cells (Gorga et al., 1984; Gorga et al., 1986; Gorga et al., 1987; Stern et al., 1992) as follows.

First, PRIESS cells (ECACC, Salisbury UK) were cultured in RPMI and 10% fetal calf serum (FCS) using standard conditions, and 10^{10} cells were lysed in 200 ml phosphate buffered saline (PBS) (pH 7.5) containing 1% NP-40 (BDH, Poole, UK), 25

mM iodoacetamide, 1 mM phenylmethylsulfonylfluoride (PMSF) and 10 mg/l each of the protease inhibitors chymostatin, antipain, pepstatin A, soybean trypsin inhibitor and leupeptin. The lysate was centrifuged at 10.000 g (30 minutes, 4°C) and the resulting supernatant was supplemented with 40 ml of an aqueous solution containing 5% sodium deoxycholate, 5 mM iodoacetamide and 10 mg/l each of the above protease inhibitors and centrifuged at 100.000 g for two hours (4°C). To remove material that bound non-specifically and endogenous antibodies, the resulting supernatant was made 0.2 mM with PMSF and passed overnight (4°C) through a rabbit serum affigel-10 column (5 ml; for preparation, rabbit serum (Charles River, Wilmington, MA, USA) was incubated with Affigel 10 (BioRad, Munich, DE) at a volume ratio of 3:1 and washed following manufacturer's directions) followed by a Protein G Sepharose Fast Flow column (2 ml; Pharmacia) using a flow rate of 0.2 ml/min.

Second, the pre-treated lysate was batch incubated with 5 ml Protein G Sepharose Fast Flow beads coupled to the murine anti-HLA-DR antibody LB3.1 (obtained by Protein G-Sepharose FF (Pharmacia) affinity chromatography of a supernatant of hybridoma cell line LB3.1) (Stern et al., 1993) overnight at 4°C using gentle mixing, and then transferred into a small column which was then washed extensively with three solutions: (1) 100 ml of a solution consisting of 50 mM Tris/HCl (pH 8.0), 150 mM NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 10% glycerol and 0.03% sodium azide at a flow rate of 0.6 ml/min). (2) 25 ml of a solution consisting of 50 mM Tris/HCl (pH 9.0), 0.5 M NaCl, 0.5 % NP-40, 0.5% sodium deoxycholate, 10% glycerol and 0.03% sodium azide at a flow rate of 0.9 ml/min; (3) 25 ml of a solution consisting of 2 mM Tris/HCl (pH 8.0), 1% octyl-ß-D-glucopyranoside, 10% glycerol and 0.03% sodium azide at a flow rate of 0.9 ml/min.

Third, MHC class II DR protein (DRA*0101/DRB1*0401) was eluted using 15 ml of a solution consisting of 50 mM diethylamine/HCl (pH 11.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% octyl-ß-D-glucopyranoside (Alexis Corp., Lausen, CH), 10% glycerol, 10 mM iodoacetamide and 0.03% sodium azide at a flow rate of 0.4 ml/min. 800 µl fractions were immediately neutralised with 100 µl 1M Tris/HCl (pH 6.8), 150 mM NaCl and 1% octyl-ß-D-glucopyranoside. The incubation of the lysate with LB3.1-Protein G Sepharose Fast Flow beads was repeated until the lysate was exhausted of

MHC protein. Pure eluted fractions of the MHC class II DR protein (as analyzed by SDS-PAGE) were pooled and concentrated to 1.0-1.3 g/l using Vivaspin concentrators (Greiner, Solingen, DE) with a 30 kDa molecular weight cut-off. Approximately 1 mg of the MHC class II DR preparation was re-buffered with PBS containing 1% octyl-β-D-glucopyranoside using the same Vivaspin concentrator to enable direct coupling of the protein to BIAcore CM5 chips.

2. Screening of HuCAL

2.1. Introduction

We identified certain antigen binding antibody fragments of human composition (MS-GPC-1, MS-GP-6, MS-GPC-8 and MS-GPC-10) against the human antigen (DRA*0101/DRB1*0401) from a human antibody library based on a novel concept that has been recently developed (Knappik et al., 2000). A consensus framework resulting in a total of 49 different frameworks here represents each of the VH- and VL-subfamilies frequently used in human immune responses. These master genes were designed to take into account and eliminate unfavorable residues promoting protein aggregation as well as to create unique restriction sites leading to modular composition of the genes. In HuCAL-scFv, both the VH- and VL-CDR3 encoding regions of the 49 master genes were randomized.

2.2. Phagemid rescue, phage amplification and purification

The HuCAL-scFv (Knappik et al., 2000) library, cloned into a phagemid-based phage display vector pMORPH13_scFv (see Figure 11), in *E.coli* TG-1 was amplified in 2 x TY medium containing 34 µg/ml chloramphenicol and 1% glucose (2 x TY-CG). After helper phage infection (VCSM13) at 37°C at an OD_{600} of about 0.5, centrifugation and resuspension in 2 x TY / 34 µg/ml chloramphenicol / 50 µg/ml kanamycin / 0.1 mM IPTG, cells were grown overnight at 30°C. Phage were PEG-precipitated from the supernatant (Ausubel et al., 1998), resuspended in PBS/20% glycerol and stored at –80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1-cells were infected with eluted phage and plated onto LB-agar supplemented with 1% of glucose and 34 µg/ml of chloramphenicol. After overnight incubation at 30°C colonies were scraped off, adjusted to an OD_{600} of 0.5 and helper phage added as described above.

2.3. Manual solid phase panning

Wells of MaxiSorp™ microtiterplates (Nunc, Roskilde, DK) were coated with MHC-class II DRA*0101/DRB1*0401 (prepared as above) dissolved in PBS (2 µg/well). After blocking with 5% non-fat dried milk in PBS, 1–5 x 10^{12} HuCAL-scFv phage purified as above were added for 1h at 20°C. After several washing steps, bound phages were eluted by pH-elution with 100 mM triethylamine and subsequent neutralization with 1M TRIS-Cl pH 7.0. Three rounds of panning were performed with phage amplification conducted between each round as described above.

2.4. Mixed solid phase/whole cell panning

Three rounds of panning and phage amplification were performed as described in 2.3. and 2.2. with the exception that in the second round between 1 x 10^7 and 5 x 10^7 PRIESS cells in 1 ml PBS/10% FCS were used in 10 ml Falcon tubes for whole cell panning. After incubation for 1h at 20°C with the phage preparation, the cell suspension was centrifuged (2000 rpm for 3 min) to remove non-binding phage, the cells were washed three times with 10 ml PBS, each time followed by centrifugation as described. Phage that specifically bound to the cells were eluted off by pH-elution using 100 mM HCl. Alternatively, binding phage could be amplified by directly adding E.coli to the suspension after triethlyamine treatment (100 mM) and subsequent neutralization.

2.5 Identification of HLA-DR binding scFv fragments

Clones obtained after three rounds of solid phase panning (2.3) or mixed solid phase/whole cell panning (2.4) were screened by FACS analysis on PRIESS cells for binding to HLA-DR on the cell surface. For expression, the scFv fragments were cloned via <u>XbaI</u>/<u>Eco</u>RI into pMx7_FS as expression vector (see Figure 12). Expression conditions are shown below in example 3.2

Aliquots of 10^6 Priess cells were transferred at 4°C into wells of a 96-well microtiterplate. ScFv in blocking buffer (PBS/5% FCS) were added for 60 min and detected using an anti-FLAG M2 antibody (Kodak) (1:5000 dilution) followed by a polyclonal goat anti-mouse IgG antibody-R-Phycoerythrin-conjugate (Jackson ImmunoResearch, West Grove, PA, USA, Cat. No. 115-116-146, $F(ab')_2$ fragment) (1:200 dilution). Cells were fixed in 4% paraformaldehyde for storage at 4°C. 10^4

events were collected for each assay on the FACS-Calibur (BD Immunocytometry Systems, San Jose, CA, USA).

Only fifteen out of over 500 putative binders were identified which specifically bound to Priess cells. These clones were further analyzed for their killing activity as described below. Table 1 contains the sequence characteristics of clones MS-GPC-1, MS-GPC-6, MS-GPC-8 and MS-GPC-10 identified thereby. The VH and VL families and the CDR3s listed refer to the HuCAL consensus-based antibody genes as described (Knappik et al., 2000); the sequences of the VH and VL CDRs are shown in Table 1, and the full sequences of the VH and VL domains are shorn in Figure 15.

3. Generation of Fab-fragments

3.1. Conversion of scFv to Fab

The Fab-fragment antigen binding polypeptides MS-GPC-1-Fab, MS-GP-6-Fab, MS-GPC-8-Fab and MS-GPC-10-Fab were generated from their corresponding scFv fragments as follows. Both heavy and light chain variable domains of scFv fragments were cloned into pMx9_Fab (Figure 13), the heavy chain variable domains as MfeI / StyI-fragments, the variable domains of the kappa light chains as EcoRV/ BsiWI-fragments. The lambda chains were first amplified from the corresponding pMORPH13_scFv vector as template with PCR-primers CRT5 (5' primer) and CRT6 (3' primer), wherein CRT6 introduces a unique DraIII restriction endonuclease site.

CRT5: 5' GTGGTGGTTCC*GATATC* 3'

CRT6: 5' AGCGTCAC*ACTCGGTG*CGGCTTTCGGCTGGCCAAGAACGGGTTA 3'

The PCR product is cut with EcoRV / DraIII and cloned into pMx9_Fab (see Figure 13). The Fab light chains could be detected with a polyclonal goat anti-human IgG antibody-R-Phycoerythrin-conjugate (Jackson ImmunoResearch, West Grove, PA, USA, Cat. No. 109-116-088, F(ab')$_2$ fragment) (1:200 dilution).

3.2. Expression and purification of HuCAL-antibody fragments in E.coli

Expression in E.coli cells (JM83) of scFv and Fab fragments from pMx7_FS or pMx9_Fab, respectively, were carried out in one litre of 2 x TY-medium supplemented

with 34 µg/ml chloramphenicol. After induction with 0.5 mM IPTG (scFv) or 0.1 mM IPTG (Fab), cells were grown at 22°C for 12 hours. Cell pellets were lysed in a French Press (Thermo Spectronic, Rochester, NY, USA) in 20 mM sodium phosphate, 0.5 M NaCl, and 10 mM imidazole (pH 7.4). Cell debris was removed by centrifugation and the clear supernatant filtered through 0.2 µm pores before subjecting it to STREP tag purification using a Streptactin matrix and purification conditions according to the supplier (IBA GmbH, Göttingen, Germany). Purification by size exclusion chromatography (SEC) was performed as described by Rheinnecker et al. (1996). The apparent molecular weights were determined by SEC with calibration standards and confirmed in some instances by coupled liquid chromatography-mass spectrometry (TopLab GmbH, Martinsried, Germany).

4. Optimization of antibody fragments

In order to optimize certain biological characteristics of the HLA-DR binding antibody fragments, one of the Fab fragments, MS-GPC-8-Fab, was used to construct a library of Fab antibody fragments by replacing the parental VL λ1 chain by the pool of all lambda chains λ 1-3 randomized in CDR3 from the HuCAL library (Knappik et al., 2000).

The Fab fragment MS-GPC-8-Fab (see 3.1) was cloned via XbaI/EcoRI from pMx9_Fab_GPC-8 into pMORPH18_Fab, a phagemid-based vector for phage display of Fab fragments, to generate pMORPH18_Fab_GPC-8 (see Figure 14). A lambda chain pool comprising a unique DraIII restriction endonuclease site (Knappik et al., 2000) was cloned into pMORPH18_Fab_GPC-8 cut with NsiI and DraIII (see vector map of pMORPH18_Fab_GPC-8 in Figure 14).

The resulting Fab optimization library was screened by two rounds of panning against MHC-class II DRA*0101/DRB1*0401 (prepared as above) as described in 2.3 with the exception that in the second round the antigen concentration for coating was decreased to 12 ng/well. FACS identified optimized clones as described above in 2.5. Seven of these clones, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18 and MS-GPC-8-27, were further characterized and showed cell killing activity as found for the starting fragment MS-GPC-8. Table 1 contains the sequence characteristics of MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9,

MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18 and MS-GPC-8-27. The VH and VL families and the CDR3s listed refer to the HuCAL consensus-based antibody genes as described (Knappik et al., 2000). The full sequences of the VH and VL domains of MS-GPC-8-6, MS-GPC-8-10, MS-GPC-8-17 and MS-GPC-8-27are shown in Figure 15.

The optimized Fab forms of the anti-HLA-DR antibody fragments MS-GPC-8-6 and MS-GPC-8-17 showed improved characteristics over the starting MS-GPC-8. For example, the EC50 of the optimized antibodies was 15-20 and 5-20 nM (compared to 20-40 nM for MS-GPC-8, where the concentration is given as the concentration of the bivalent cross-linked Fab dimer), and the maximum capacity to kill MHH-Call 4 cells determined as 76 and 78% for MS-GPC-8-6 and MS-GPC-8-17 (compared to 65% for MS-GPC-8) respectively.

For further optimization, the VL CDR1 regions of a set of anti-HLA-DR antibody fragments derived from MS-GPC-8 (including MS-GPC-8-10 and MS-GPC-8-27) were optimized by cassette mutagenesis using trinucleotide-directed mutagenesis (Virnekäs et al., 1994). In brief, a Vl1 CDR1 library cassette was synthesized containing six randomized positions (total variability: 7.43×10^6), and was cloned into a Vl1 framework. The CDR1 library was digested with EcoRV and BbsI, and the fragment comprising the CDR1 library ligated into the lambda light chains of the MS-GPC-8-derived Fab antibody fragments in pMORPH18_Fab (as described above), digested with EcoRV and BbsI. The resulting library was screened as described above. Ten clones were identified as above by binding specifically to HLA DR (MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 & MS-GPC-8-27-41) and showed cell killing activity as found for the starting fragments MS-GPC-8, MS-GPC-8-10 and MS-GPC-8-27. Table 1 contains the sequence characteristics of MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 & MS-GPC-8-27-41. The VH and VL families and the CDR3s listed refer to the HuCAL consensus-based antibody genes as described (Knappik et al., 2000), the full sequences of the VH and VL domains of MS-GPC-8-6-13, MS-GPC-8-10-57 & MS-GPC-8-27-41 are shown in Figure 15.

From these 10 clones, four Fab fragments were chosen (MS-GPC-8-6-2, MS-GPC-8-6-13, MS-GPC-8-10-57 and MS-GPC-8-27-41) as demonstrating significantly improved EC50 of cell killing as described in example 10. Table 1 shows the sequences of clones optimised at the CDR1 region.

Optimisation procedures not only increased the biological efficacy of anti-HLA DR antibody fragments generated by the optimisation process, but a physical characteristic - affinity of the antibody fragment to HLA DR protein - was also substantially improved. For example, the affinity of Fab forms of MS-GPC-8 and its optimised descendents was measured using a surface plasmon resonance instrument (Biacore, Upsala Sweden) according to example 7. The affinity of the MS-GPC-8 parental Fab was improved over 100 fold from 346 nM to ~ 60 nM after VLCDR3 optimisation and further improved to single digit nanomolar affinity (range 3 – 9 nM) after VLCDR3+1 optimisation (Table 2).

5. Generation of IgG

5.1 Construction of HuCAL-immunoglobulin expression vectors

Heavy chains were cloned as follows. The multiple cloning site of pcDNA3.1+ (Invitrogen) was removed (<u>Nhe</u>I / <u>Apa</u>I), and a stuffer compatible with the restriction sites used for HuCAL-design was inserted for the ligation of the leader sequences (<u>Nhe</u>I / <u>Eco</u>RI), VH-domains (<u>Eco</u>RI / <u>Blp</u>I) and the immunoglobulin constant regions (<u>Blp</u>I / <u>Apa</u>I). The leader sequence (EMBL M83133) was equipped with a Kozak sequence (Kozak, 1987). The constant regions of human IgG1 (PIR J00228), IgG4 (EMBL K01316) and serum IgA1 (EMBL J00220) were dissected into overlapping oligonucleotides with lengths of about 70 bases. Silent mutations were introduced to remove restriction sites non-compatible with the HuCAL-design. The oligonucleotides were spliced by overlap extension-PCR.

Light chains were cloned as follows. The multiple cloning site of pcDNA3.1/Zeo+ (Invitrogen) was replaced by two different stuffers. The κ-stuffer provided restriction sites for insertion of a κ-leader (<u>Nhe</u>I / <u>Eco</u>RV), HuCAL-scFv Vκ-domains (<u>Eco</u>RV / <u>Bsi</u>WI) and the κ-chain constant region (<u>Bsi</u>WI / <u>Apa</u>I). The corresponding restriction

sites in the λ-stuffer were Nhel / EcoRV (λ-leader), EcoRV / Hpal (Vλ- domains) and Hpal / Apal (λ-chain constant region). The κ-leader (EMBL Z00022) as well as the λ-leader (EMBL L27692) were both equipped with Kozak sequences. The constant regions of the human κ- (EMBL J00241) and λ -chain (EMBL M18645) were assembled by overlap extension-PCR as described above.

5.2 Generation of IgG-expressing CHO-cells

All cells were maintained at 37°C in a humidified atmosphere with 5% CO2 in media recommended by the supplier. CHO-K1 (CRL-9618) were from ATCC and were co-transfected with an equimolar mixture of IgG heavy and light chain expression vectors. Double-resistant transfectants were selected with 600 µg/ml G418 and 300 µg/ml Zeocin (Invitrogen) followed by limiting dilution. The supernatant of single clones was assessed for IgG expression by capture-ELISA. Positive clones were expanded in RPMI-1640 medium supplemented with 10% ultra-low IgG-FCS (Life Technologies). After adjusting the pH of the supernatant to 8.0 and sterile filtration, the solution was subjected to standard protein A column chromatography (Poros 20A, PE Biosystems).

The IgG forms of anti-HLA-DR antigen binding domains show improved characteristics over the antibody fragments. These improved characteristics include affinity (Example 7) and killing efficiency (Examples 9, 10 and 14).

6. HLA-DR specificity assay and epitope mapping

To demonstrate that antigen-binding domains selected from the HuCAL library bound specifically to a binding site on the N-terminal domain of human MHCII receptor largely conserved between alleles and hitherto unknown in the context of cell killing by receptor cross linking, we undertook an assessment of their binding specificity, and it was attempted to characterise the binding epitope.

The Fab antibody fragments MS-GPC-8-27-7, MS-GPC-8-27-10, MS-GPC-8-6-13, MS-GPC-8-27-41, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-6-27, MS-GPC-8 and MS-GPC-8-6 showed specificity of binding to HLA-DR protein but not to non-HLA-DR proteins. Fab fragments selected from the HuCAL library were tested for reactivity with the following antigens: HLA-DR protein (DRA*0101/DRB1*0401;

prepared as example 1, and a set of unrelated non-HLA-DR proteins consisting of BSA, testosterone-BSA, lysozyme and human apotransferrin. An empty well (Plastic) was used as negative control. Coating of the antigen MHCII was performed over night at 1 µg/well in PBS (Nunc-MaxiSorp TM) whereas for the other antigens (BSA, Testosterone-BSA, Lysozyme, Apotransferrin) 10 µg/well was used. Next day wells were blocked in 5% non-fat milk for 1 hr followed by incubation of the respective antibodies (anti-MHCII-Fabs and an unrelated Fab (Mac1-8A)) at 100 ng/well for 1h. After washing in PBS the anti-human IgG F(ab')2-peroxidase-conjugate at a 1:10000 dilution in TBS (supplemented with 5% w/v non-fat dry-milk/0.05% v/v Tween 20) was added to each well for 1h. Final washes were carried out in PBS followed the addition the substrate POD (Roche). Color-development was read at 370 nM in an ELISA-Reader

All anti-HLA-DR antibody fragments MS-GPC-8-27-7, MS-GPC-8-27-10, MS-GPC-8-6-13, MS-GPC-8-27-41, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-6-27, MS-GPC-8 and MS-GPC-8-6 demonstrated high specificity for HLA-DR, as evidenced by the much higher mean fluorescence intensity resulting from incubation of these antibody fragments with HLA-DR derived antigens compared to controls (Figure 1a). In a similar experiment, the Fab fragments MS-GPC-1, MS-GPC-6, MS-GPC-8 and MS-GPC-10 were found to bind to both the DRA*0101/DRB1*0401 (preparaed as above) as well as to a chimeric DR-IE consisting of the N-terminal domains of DRA*0101 and DRB1*0401 with the remaining molecule derived from a murine class II homologue IEd (Ito et al., 1996) (Figure 1b).

To demonstrate the broad-DR reactivity of anti-HLA-DR antibody fragments and IgGs of the invention, the scFv forms of MS-GPC-1, 6, 8 and 10, and IgG forms of MS-GPC-8, MS-GPC-8-10-57, MS-GPC-8-27-51 & MS-GPC-8-6-13 were tested for reactivity against a panel of Epstein-Barr virus transformed B cell lines obtained from ECACC (Salisbury UK), each homozygous for one of the most frequent DR alleles in human populations (list of cell lines and alleles shown in Figure 2). The antibody fragments were also tested for reactivity against a series of L cells transfected to express human class II isotypes other than DRB1: L105.1, L257.6, L25.4, L256.12 & L21.3 that express the molecules DRB3*0101, DRB4*0101, DP0103/0402, DP 0202/0201, and DQ0201/0602 respectively (Klohe et al., 1988).

Reactivity of an antigen-binding fragment to the panel of cell-lines expressing various MHC- class II molecules was demonstrated using an immunofluorescence procedure as for example, described by Otten et al (1997). Staining was performed on $2x10^5$ cells using an anti-FLAG M2 antibody as the second reagent against the M2 tag carried by each anti-HLA-DR antibody fragment and a fluorescein labelled goat anti-mouse Ig (BD Pharmingen, Torrey Pine, CA, USA) as a staining reagent. Cells were incubated at 4°C for 60 min with a concentration of 200 nM of the anti-HLA-DR antibody fragment, followed by the second and third antibody at concentrations determined by the manufacturers. For the IgG form, the second antibody was omitted and the IgG detected using a FITC-labeled mouse anti-human IgG4 (Serotec, Oxford, UK) . Cells were washed between incubation steps. Finally the cells were washed and subjected to analysis using a FACS Calibur (BD Immunocytometry Systems, San Jose, CA, USA).

Figure 2 shows that the scFv-fragments MS-GPC-1, 6, 8 and 10, and IgG forms of MS-GPC-8, MS-GPC-8-10-57, MS-GPC-8-27-51 & MS-GPC-8-6-13 react with all DRB1 allotypes tested. This observation taken together with the observation that all anti-HLA-DR antibody fragments react with chimeric DR-IE, suggests that all selected anti-HLA-DR antibody fragments recognize the extracellular first domain of the monomorphic DRα chain or a monomorphic epitope on extracellular first domain of the DRβ chain.

We then attempted to localize the binding domains of MS-GPC-8-10-57 and MS-GPC-8-27-41 further by examining competitive binding with murine antibodies for which the binding domains on HLA-DR are known. The murine antibodies L243 and LB3.1 are known to bind to the α1 domain, 1-1C4 and 8D1 to the β1 domain and 10F12 to the β2 domain (Vidovic et al. 1995b). To this end, an assay was developed wherein a DR-expressing cell line (LG-2) was at first incubated with the IgG4 forms of MS-GPC-8-10-57 or MS-GPC-8-27-41, the Fab form of MS-GPC-8-10-57 or the Fab form of GPC 8, and an unrelated control antibody. Subsequently murine antibodies were added and the murine antibodies were detected. If the binding site of MS-GPC-8-10-57 or MS-GPC-8-27-41 overlaps with the binding of a murine antibody, then a reduced detection of the murine antibody is expected.

Binding of the IgG4 forms of GPC-8-27-41 and MS-GPC-8-10-57 and the Fab form of MS-GPC-8-10-57 substantially inhibited (mean fluorescence intensity reduced by > 90%) the binding of 1-1C4 and 8D1, whereas L243, LB3.1 and 10F12 and a control were only marginally affected. The Fab form of MS-GPC-8 reduced binding of 1-1C4 by ~ 50% (mean fluorescence dropped from 244 to 118), abolished 8D1 binding and only marginally affected binding of L243, LB3.1 and 10F12 or the control. An unrelated control antibody had no effect on either binding. Thus, MS-GPC-8-10-57 and MS-GPC-8-27-41 seem to recognise a β1 domain epitope that is highly conserved among allelic HLA-DR molecules.

The whole staining procedure was performed on ice. 1x 10^7 cells of the human B-lymphoblastoid cell line LG-2 was preblocked for 20 Min. in PBS containing 2% FCS and 35 µg/ml Guinea Pig IgG ("FACS-Buffer"). These cells were divided into 3 equal parts A, B, and C of approximately 3.3 x 10^6 cells each, and it was added to A.) 35 µg MS-GPC-8-10-57 or MS-GPC-8-27-41 IgG4, to B.) 35 µg MS-GPC-8-10-57 Fab or MS-GPC-8 Fab, and to C.) 35 µg of an unrelated IgG4 antibody as negative control, respectively, and incubated for 90 min. Subsequently A, B, C were divided in 6 equal parts each containing 5.5 x 10^5 cells, and 2 µg of the following murine antibodies were added each to one vial and incubated for 30 min: 1.) purified mIgG ; 2.) L243; 3.) LB3.1; 4.) 1-1 C4; 5.) 8D1; 6.) 10F12. Subsequently, 4ml of PBS were added to each vial, the vials were centrifuged at 300g for 8 min, and the cell pellet resuspended in 50 µl FACS buffer containing a 1 to 25 dilution of a goat-anti-murine Ig-FITC conjugate at 20 µg/ml final concentration (BD Pharmingen, Torrey Pines, CA, USA). Cells were incubated light-protected for 30 min. Afterwards, cells were washed with 4 ml PBS, centrifuged as above and resuspended in 500 µl PBS for analysis in the flow cytometer (FACS Calibur, BD Immunocytometry Systems, San Jose, CA, USA).

The PepSpot technique (US 6040423; Heiskanen et al., 1999) is used to further identify the binding epitope for MS-GPC 8-10-57. Briefly, an array of 73 overlapping 15mer peptides is synthesised on a cellulose membrane by a solid phase peptide synthesis spotting method (WO 00/12575). These peptide sequences are derived from the sequence of the α1 and ß1 domains of HLA-DR4Dw14, HLA-DRA1*0101 (residues 1-81) and HLA-DRB1*0401 (residues 2-92), respectively, and overlap by

two amino acids. Second, such an array is soaked in 0.1% Tween-20/PBS (PBS-T), blocked with 5% BSA in PBS-T for 3 hours at room temperature and subsequently washed three times with PBS-T. Third, the prepared array is incubated for 90 minutes at room temperature with 50 ml of a 5 mg/l solution of the IgG form of GPC-8-10-57 in 1% BSA/PBS-T. Fourth, after binding, the membrane is washed three times with PBS-T and subsequently incubated for 1 hour at room temperature with a goat anti-human light chain antibody conjugated to horseradish peroxidase diluted 1/5000 in 1% BSA/PBS-T. Finally, the membrane is washed three times with PBS-T and any binding determined using chemiluminescence detection on X-ray film. As a control for unspecific binding of the goat anti-human light chain antibody, the peptide array is stripped by the following separate washings each at room temperature for 30 min: PBS-T (2 times), water, DMF, water, an aequeous solution containing 8M urea, 1% SDS, 0.5% DTT, a solution of 50% ethanol, 10% acetic acid in water (3 times each) and, finally, methanol (2 times). The membrane is again blocked, washed, incubated with goat anti-human l light chain antibody conjugated to horseradish peroxidase and developed as described above.

7. Affinity of anti- HLA-DR antibody and antibody fragments

In order to demonstrate the superior binding properties of anti-HLA antibody fragments of the invention, we measured their binding affinities to the human MHC class II DR protein (DRA*0101/DRB1*0401) using standard equipment employing plasmon resonance principles. Surprisingly, we achieved affinities in the sub-nanomolar range for IgG forms of certain anti-HLA-DR antibody fragments of the invention. For example, the affinity of the IgG forms of MS-GPC-8-27-41, MS-GPC-8-6-13 & MS-GPC-8-10-57 was measured as 0.3, 0.5 and 0.6 nM respectively (Table 3a). Also, we observed high affinities in the range of 2-8 nM for Fab fragments affinity matured at the CDR1 and CDR3 light chain regions (Table 3b). Fab fragments affinity matured at only the CDR3 light chain region showed affinities in the range of 40 to 100 nM (Table 3c), and even Fab fragments of non-optimised HuCAL antigen binding domains showed affinities in the sub μM range (Table 3d). Only a moderate increase in Kon (2-fold) was observed following CDR3 optimisation (Kon remained approximately constant throughout the antibody optimization process in the order of 1 x 10^5 $M^{-1}s^{-1}$), whilst a significant decrease in Koff was a surprising feature of the optimisation process – sub 100 s^{-1}, sub 10 s^{-1}, sub 1 s^{-1} and sub 0.1 s^{-1} for the

unoptimised Fabs, CDR3 optimised Fabs, CDR3/CDR1 optimised Fabs and IgG forms of anti-HLA-DR antibody fragments of the invention.

The affinities for anti-HLA antibody fragments of the invention were measured as follows. All measurements were conducted in HBS buffer (20mM HEPES, 150mM NaCl, pH7.4) at a flow rate of 20µl/min at 25°C on a BIAcore3000 instrument (Biacore AB, Sweden). MHC class II DR protein (prepared as example 1) was diluted in 100mM sodium acetate pH 4.5 to a concentration of 50 - 100 mg/ml, and coupled to a CM5 chip (Biacore AB) using standard EDC-NHS coupling chemistry with subsequent ethanolamine treatment as manufacturers directions. The coating density of MHCII was adjusted to between 500 and 4000 RU. Affinities were measured by injection of 5 different concentrations of the different antibodies and using the standard software of the Biacore instrument. Regeneration of the coupled surface was achieved using 10mM glycine pH2.3 and 7.5mM NaOH.

8. Multivalent killing activity of anti HLA-DR antibodies and antibody fragments

To demonstrate the effect of valency on cell killing, a cell killing assay was performed using monovalent, bivalent and multivalent compositions of anti-HLA-DR antibody fragments of the invention against GRANTA-519 cells. Anti-HLA-DR antibody fragments from the HuCAL library showed much higher cytotoxic activity when cross-linked to form a bivalent composition (60 – 90% killing at antibody fragment concentration of 200 nM) by co-incubation with anti-FLAG M2 mAb (Figure 3) compared to the monovalent form (5 – 30% killing at antibody fragment concentration of 200 nM). Incubation of cell lines alone or only in the presence of anti-FLAG M2 mAb without co-incubation of anti-HLA-DR antibody fragments did not lead to cytotoxicity as measured by cell viability. Treatment of cells as above but using 50 nM of the IgG4 forms (naturally bivalent) of the antibody fragments MS-GPC-8, MS-GPC-8-6-13, MS-GPC-8-10-57 and MS-GPC-8-27-41 without addition of anti-FLAG M2 mAb showed a killing efficiency after 4 hour incubation of 76%, 78%, 78% and 73% respectively.

Furthermore, we observed that higher order valences of the anti-HLA-DR antibody fragments further decrease cell viability significantly. On addition of Protein G to the incubation mix containing the IgG form of the anti-HLA-DR antibody fragments, the

multivalent complexes thus formed further decrease cell viability compared to the bivalent composition formed from incubation of the anti-HLA-DR antibody fragments with only the bivalent IgG form.

The killing efficiency of anti-HLA-DR antibody fragments selected from the HuCAL library was tested on the HLA-DR positive tumor cell line GRANTA-519 (DSMZ, Germany). $2x10^5$ cells were incubated for 4 h at 37°C under 6% CO_2 with 200 nM anti-HLA-DR antibody fragments in RPMI 1640 (PAA, Germany) supplemented with 2,5% heat inactivated FBS (Biowhittaker Europe, BE), 2mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate and 0,1 mg/ml kanamycin. Each anti-HLA-DR antibody fragment was tested for its ability to kill activated tumor cells as a monovalent anti-HLA-DR antibody fragment or as a bivalent composition by the addition of 100 nM of a bivalent cross-linking anti-FLAG M2 mAb. After 4 h incubation at 37°C under 6% CO_2, cell viability was determined by trypan blue staining and subsequent counting of remaining viable cells (Current Protocols in Immunology, 1997).

The above experiment was repeated using KARPAS-422cells against a multivalent form of IgG forms of MS-GPC-8-10-57 and MS-GPC-8-27-41 prepared by a pre-incubation with a dilution series of the bacterial protein Protein G. Protein G has a high affinity and two binding sites for IgG antibodies, effectively cross-linking them to yield a total binding valency of 4. In a control using IgG alone without preincubation with Protein G, approximately 55% of cells were killed, while cell killing using IgG pre-incubated with Protein G gave a maximum of approximately 75% at a molar ratio of IgG antibody/Protein G of ~ 6 (based on a molecular weight of Protein G of 28.5 kD). Higher or lower molar ratios of IgG antibody/Protein G approached the cell killing efficiency of the pure IgG antibodies.

9. Killing efficiency of anti-HLA-DR antibody fragments

Experiments to determine the killing efficiency of the anti-HLA-DR cross-linked antibody fragments against other tumor cell lines that express HLA-DR molecules were conducted analogous to example 8. Tumor cell lines that show greater than 50% cell killing with the cross linked Fab form of MS-GPC-8 after 4 h incubation include MHH-CALL4, MN 60, BJAB, BONNA-12 which represent the diseases B cell acute

lymphoid leukemia, B cell acute lymphoid leukemia, Burkitt lymphoma and hairy cell leukemia respectively. Use of the cross-linked Fab form of the anti-HLA-DR antibody fragments MS-GPC-1, 6 and 10 also shows similar cytotoxic activity to the above tumor cell lines when formed as a bivalent agent using the cross-linking anti-FLAG M2 mAb.

The method described in example 8 was used to determine the maximum killing capacity for each of the cross-linked bivalent anti-HLA-DR antibody fragments against Priess cells. The maximum killing capacity observed for MS-GPC-1, MS-GPC-6, MS-GPC-8 & MS-GPC-10 was measured as 83%, 88%, 84% and 88% respectively. Antibody fragments generated according to example 4, when cross linked using anti-FLAG M2 mAb as above, also showed improved killing ability against GRANTA and Priess cells (Table 4).

10. Killing efficiency of anti-HLA-DR IgG antibodies of human composition

Compared to corresponding murine antibodies (Vidovic et al, 1995b; Nagy & Vidovic, 1996; Vidovic & Toral; 1998), we were surprised to observe significantly improved killing efficiency of IgG forms of certain anti-HLA-DR antibody fragments of the invention (Table 5). Following the method described in examples 8 and 9 but at 50 nM, repeated measurements (3 to 5 replica experiments where cell number was counted in duplicate for each experiment) were made of the killing efficiency of the IgG forms of certain antibody fragments of the invention. When applied at a final concentration of only 50 nM, IgGs of the antibody fragments MS-GPC-8, MS-GPC-8-6-13, MS-GPC-8-10-57 & MS-GPC-8-27-41 killed more than 50% of cells from 16, 22, 19 and 20 respectively of a panel of 24 human tumor cell lines that express HLA-DR antigen at a level greater than 10 fluorescent units as determined by example 11. Cells were treated with the two murine anti-HLA-DR antibodies L243 (Vidovic et al, 1995b) and 8D1 (Vidovic & Toral; 1998) at a significantly higher final concentration of mAb (200 nM), which reduced cell viability to a level below 50% viable cells in only 13 and 12 of the 24 HLA-DR expressing cells lines, respectively. The cell line MHH-PREB-1 was singled out and not accounted as part of the panel of 24 cell lines despite its expression of HLA-DR antigen at a level greater than 10 fluorescent units due to the inability of any of the above antibodies to induce any significant reduction of cell viability. This is further explained in example 12.

Indeed, even at the significantly increased concentration, the two murine antibodies treated at 200 nM showed significantly less efficient killing compared to the IgG forms of anti-HLA DR antibody fragments of the invention. Not only do IgG forms of the human anti-HLA-DR antibody fragments of the invention show an overall increase in cell killing at lower concentrations compared to the murine antibodies, but they show less variance in killing efficiency across different cell lines.The coefficient of variance in killing for the human antibodies in this example is 32% (mean %killing = 68 +/- 22% (SD)), compared to over 62% (mean %killing = 49 +/- 31% (SD)) for the mouse antibodies. Statistically controlling for the effect on killing efficiency due to HLA expression by fitting logistic regression models to mean percentage killing against log(mean HLA DR expression) supports this observation (Figure 4). Not only is the fitted curve for the murine antibodies consitently leower than that for the human, but a larger variance in residuals from the murine antibody data (SD = 28%) is seen compared to the variance in residuals from the human antibody data (16%).

11. Killing selectivity of antigen-binding domains against a human antigen for activated versus non-activated cells

Human peripheral B cells were used to demonstrate that human anti-HLA-DR mAb-mediated cell killing is dependent on cell-activation. 50 ml of heparinised venous blood was taken from an HLA-DR typed healthy donor and fresh peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque Gradient Centrifugation (Histopaque-1077; Sigma) as described in Current Protocols in Immunology (John Wiley & Sons, Inc.; 1999). Purified B cells (~5% of peripheral blood leukocytes) were obtained from around $5x10^7$ PBMC using the B-cell isolation kit and MACS LS^+/VS^+ columns (Miltenyi Biotec, Germany) according to manufacturers guidelines. Successful depletion of non-B cells was verified by FACS analysis of an aliquot of isolated B cells (HLA-DR positive and CD19 positive). Double staining and analysis is done with commercially available antibodies (BD Immunocytometry Systems, San Jose, CA, USA) using standard procedures as for example described in Current Protocols in Immunology (John Wiley & Sons, Inc.; 1999). An aliquot of the isolated B cells was tested for the ability of the cells to be activated by stimulation with Pokeweed mitogen (PWM) (Gibco BRL, Cat. No. 15360-019) diluted 1:25 in RPMI 1640 (PAA, Germany) supplemented with 10% FCS (Biowhittaker Europe, BE), 2mM

L-glutamine, 1% non-essential amino acids, 1mM sodium pyruvate and 0,1mg/ml kanamycin by incubation at 37°C under 6% CO_2 for three days. Successful activation was verified by FACS analysis of HLA-DR expression on the cell surface (Current Protocols in Immunology, John Wiley & Sons, Inc.; 1999).

The selectivity for killing of activated cells versus non-activated cells was demonstrated by incubating $1x10^6$/ml B cells activated as above compared to non-activated cells, respectively with 50 nM of the IgG forms of MS-GPC-8-10-57, MS-GPC-8-27-41 or the murine IgG 10F12 (Vidovic et al., 1995b) in the medium described above but supplemented with 2,5% heat inactivated FCS instead of 10%, or with medium alone. After incubation at 37°C under 6% CO_2 for 1 or 4h, cell viability was determined by fluorescein diacetate staining (FDA) of viable and propidium iodide staining (PI) of dead cells and subsequent counting of the green (FDA) and red (PI) fluorescent cells using a fluorescence microscope (Leica, Germany) using standard procedures (Current Protocols in Immunology, 1997).

B cell activation was shown to be necessary for cell killing. In non-activated cells after 1 h of incubation with the anti-HLA-DR antibodies, the number of viable cells in the media corresponded to 81%, 117% 126% and 96% of the pre-incubation cell density for MS-GPC-8-10-57 (IgG), MS-GPC-8-27-41 (IgG), 10F12 and medium alone, respectively. In contrast, the number of viable activated B cells after 1 h incubation corresponded to 23%, 42% 83% and 66% of the pre-incubation cell density for MS-GPC-8-10-57 (IgG), MS-GPC-8-27-41 (IgG), 10F12 and medium alone, respectively. After 4 h of incubation, 78%, 83% 95% and 97% of the pre-incubation cell density for MS-GPC-8-10-57 (IgG), MS-GPC-8-27-41 (IgG), 10F12 and medium alone were found viable in non-activated cells, whereas the cell density had dropped to 23%, 24% 53% and 67% of the pre-incubation cell density for MS-GPC-8-10-57 (IgG), MS-GPC-8-27-41 (IgG), 10F12 and medium alone, respectively, in activated cells.

12. Killing activity of anti-HLA antibody fragments against the cell line MHH PreB 1

As evidenced in Table 5, we observed that our cross-linked anti-HLA-DR antibody fragments or IgGs did not readily kill a particular tumor cell line expressing HLA-DR at significant levels. We hypothesized that although established as a stable cell line, cells in this culture were not sufficiently activated. Therefore, we conducted an

experiment to stimulate activity of the MHH preB1 cell line, using increased cell-surface expression of HLA-DR molecule as a marker of activation as follows.

Non-adherently growing MHH preB1 cells were cultivated in RPMI medium containing the following additives (all from Gibco BRL and Bio Whittaker): 10% FCS, 2 mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate and 1x Kanamycin. Aliquots were activated to increase expression of HLA-DR molecule by incubation for one day with Lipopolysaccharide (LPS, 10 µg/ml), Interferon-gamma (IFN-γ, Roche, 40 ng/ml) and phyto-hemagglutinin (PHA, 5 µg/ml). The cell surface expression of HLA-DR molecules was monitored by flow cytometry with the FITC-conjugated mAb L243 (BD Immunocytometry Systems, San Jose, CA, USA). Incubation of MHH preB1 for one day in the presence of LPS, IFN- γ and PHA resulted in a 2-fold increase in HLA-DR surface density (mean fluorescence shift from 190 to 390). Cell killing was performed for 4 h in the above medium but containing a reduced FCS concentration (2.5%). A concentration series of the IgG forms of MS-GPC-8-27-41 & MS-GPC-8-10-57 was employed, consisting of final antibody concentrations of 3300, 550, 92, 15, 2.5, 0.42 and 0.07 nM, on each of an aliquot of non-activated and activated cells. Viable cells were identified microscopically by exclusion of Trypan blue. Whereas un-activated cell viability remains unaffected by the antibody up to the highest antibody concentration used, cell viability is dramatically reduced with increasing antibody concentration in activated MHH PreB1 cells (Figure 5).

13. Killing efficiency of anti-HLA-DR IgG antibodies of human composition against ex-vivo chronic lymphoid leukemia cells

Using B cells isolated and purified from 10 patients suffering from chronic lymphoid leukemia (CLL), we demonstrated that IgG forms of anti-HLA-DR antibody fragments of the invention showed efficacy in killing of clinically relevant cells using an <u>ex-vivo</u> assay. B-cells were isolated and purified from 10 unrelated patients suffering from CLL (samples kindly provided by Prof Hallek, Ludwig Maximillian University, Munich) according to standard procedures (Buhmann et al., (1999)). $2x10^5$ cells were treated with 100 nM of IgG forms of the anti-HLA-DR antibody fragments MS-GPC-8, MS-GPC-8-10-57 or MS-GPC-8-27-41 and incubated for 4 or 24 hours analogous to examples 8 and 9. A replica set of cell cultures was established and activated by

incubation with HeLa-cells expressing CD40 ligand on their surface for three days before treatment with antibody (Buhmann et al., 1999). As controls, the murine IgG 10F12 (Vidovic et al., 1995b) or no antibody was used. Cell viability for each experiment was determined as described in example 12.

Surprisingly, IgG forms of the anti-HLA-DR antibody fragments of the invention showed highly efficient and uniform killing - even across this diverse set of patient material. After only 4 hours of treatment, all three human IgGs gave a significant reduction in cell viability compared to the controls, and after 24 hours only 33% of cells remained viability (Figure 6). We found that on stimulating the ex-vivo cells further according to Buhmann et al (1999), the rate of killing was increased such that after only 4 hours culture with the human antibodies, only 24% of cells remained viable on average for all patient samples and antibody fragments of the invention.

14. Determination of EC50 for anti-HLA-DR antibody fragments

We demonstrated superior Effective Concentration at 50% effect (EC50) values in a cell-killing assay for certain forms of anti-HLA-DR antibody fragments selected from the HuCAL library compared to cytotoxic murine anti-HLA-DR antibodies (Table 6).

The EC50 for anti-HLA-DR antibody fragments selected from the HuCAL library were estimated using the HLA-DR positive cell line PRIESS or LG2 (ECACC, Salisbury UK). $2x10^5$ cells were incubated for 4 h at 37°C under 6% CO_2 in RPMI 1640 (PAA, Germany) supplemented with 2,5% heat inactivated FBS (Biowhittaker Europe, BE), 2mM L-glutamine, 1% non-essential amino acids, 1mM sodium pyruvate and 0,1mg/ml kanamycin, together with dilution series of bivalent anti-HLA-DR antibody fragments. For the dilution series of Fab antibody fragments, an appropriate concentration of Fab fragment and anti-FLAG M2 antibody were premixed to generate bivalent compositions of the anti-HLA-DR antibody fragments. The concentrations stated refer to the concentration of bivalent composition such that the IgG and Fab EC50 values can be compared.

After 4 h incubation with bivalent antibody fragments at 37°C under 6% CO_2, cell viability was determined by fluorescein diacetate staining and subsequent counting of remaining viable cells (Current Protocols in Immunology, 1997). Using standard

statistical software, non-linear logistic regression curves were fitted to replica data points and the EC50 estimated for each antibody fragment.

When cross-linked using the anti-FLAG M2 antibody, the Fab fragments MS-GPC-1, MS-GPC-8 & MS-GPC-10 selected from the HuCAL library (Example 4) showed an EC50 of less than 120 nM as expressed in terms of the concentration of the monovalent fragments, which corresponds to a 60 nM EC50 for the bivalent cross-linked (Fab)dimer-anti-Flag M2 conjugate. (Figure 7a). When cross-linked using the anti-FLAG M2 antibody, anti-HLA-DR antibody fragments optimised for affinity within the CDR3 region (Example 4) showed a further improved EC50 of less than 50 nM, or 25 nM in terms of the bivalent cross-linked fragment (Figure 7b), and those additionally optimised for affinity within the CDR1 region showed an EC50 of less than 30 nM (15 nM for bivalent fragment). In comparison, the EC50 of the cytotoxic murine anti-HLA-DR antibodies 8D1 (Vidovic & Toral; 1998) and L243 (Vidovic et al; 1995b) showed an EC50 of over 30 and 40 nM, respectively, within the same assay (Figure 7c).

Surprisingly, the IgG form of certain antibody fragments of the invention showed approximately 1.5 orders of magnitude improvement in EC50 compared to the murine antibodies (Figure 7d). For example, the IgG forms of MS-GPC-8-10-57 & MS-GPC-8-27-41 showed an EC50 of 1.2 and 1.2 nM respectively. Furthermore, despite being un-optimised for affinity, the IgG form of MS-GPC-8 showed an EC50 of less than 10 nM.

As has been shown in examples 11 and 12, the efficiency of killing of un-activated cells (normal peripheral B and MHH PreB cells respectively) is very low. After treatment with 50 nM of the IgG forms of MS-GPC-8-10-57 & MS-GPC-8-27-41, 78% and 83% of normal peripheral B cells, respectively, remain viable after 4 hours. Furthermore, at only 50nM concentration or either IgG, virtually 100% viability is seen for MHH PreB1 cells. Indeed, a decrease in the level of viability to below 50% cannot be achieved with these un-activated cells using reasonable concentration ranges (0.1 to 300 nM) of IgG or bivalent cross-linked Fab forms of the anti-HLA DR antibody fragments of the invention. Therefore, the EC50 for these un-activated cell types can be estimated to be at least 5 times higher than that shown for the non-optimised Fab

forms (EC50 ~ 60 nM with respect to cross-linked bivalent fragment), and at least 10 times and 100 times higher than EC50s shown for the VHCDR3 optimised Fabs (~ 25 nM with respect to cross-linked bivalent fragment) and IgG forms of MS-GPC-8-10-57 (~1.2 nM) & MS-GPC-8-27-41 (~1.2 nM) respectively.

15. Mechanism of cell-killing

The examples described above show that cell death occurs - needing only certain multivalent anti-HLA-DR antibody fragments to cause killing of activated cells. No further cytotoxic entities or immunological mechanisms were needed to cause cell death, therefore demonstrating that cell death is mediated through an innate pre-programmed mechanism of the activated cell. The mechanism of apoptosis is a widely understood process of pre-programmed cell death. We were surprised by certain characteristics of the cell killing we observed that suggested the mechanism of killing for activated cells when exposed to our human anti-HLA-DR antibody fragments was not what is commonly understood in the art as "apoptosis". For example, the observed rate of cell killing appeared to be significantly greater than the rate reported for apoptosis of immune cells (about 10 - 15 h; Truman et al., 1994). Two experiments were conducted to demonstrate that the mechanism of cell killing proceeded by a non-apoptotic mechanism.

First, we used Annexin-V-FITC and propidium iodide (PI) staining techniques to distinguish between apoptotic and non-apoptotic cell death – cells undergoing apoptosis, "apoptotic cells", (Annexin-V positive/PI negative) can be distinguished from necrotic ("Dead") (Annexin-V positive/PI positive) and fully functional cells (Annexin-V negative/PI negative). Using the procedures recommended by the manufacturers of the AnnexinV and PI assays, 1x10^6/ml Priess cells were incubated at 37°C under 6% CO_2 with or without 200 nM anti-HLA-DR antibody fragment MS-GPC-8 together with 100 nM of the cross-linking anti-FLAG M2 mAb in RPMI 1640 (PAA, DE) supplemented with 2,5% heat inactivated FCS (Biowhittaker Europe, BE), 2mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate and 0,1 mg/ml kanamycin. To provide an apoptotic cell culture as control, 1x10^6/ml Priess cells were induced to enter apoptosis by incubation in the above medium at 37°C under 6% CO_2 with 50 μg/ml of the apoptosis-inducing anti-CD95 mAb DX2 (BD Pharmingen, Torrey Pine, CA, USA) cross-linked with 10 μg/ml Protein-G. At various

incubation times (1, 15 and 60 min, 3 and 5 h) 200 µl samples were taken, washed twice and stained with Annexin-V-FITC (BD Pharmingen, Torrey Pine, CA, USA) and PI using Annexin-V binding buffer following the manufacturer's protocol. The amount of staining with Annexin-V-FITC and PI for each group of cells is analysed with a FACS Calibur (BD Immunocytometry Systems, San Jose, CA, USA).

Cell death induced through the cross-linked anti-HLA-DR antibody fragments shows a significantly different pattern of cell death than that of the anti-CD95 apoptosis inducing antibody or the cell culture incubated with anti-FLAG M2 mAb alone. The percentage of dead cells (as measured by Annexin-V positive/PI positive staining) for the anti-HLA-DR antibody fragment/anti-FLAG M2 mAb treated cells increases far more rapidly than that of the anti-CD95 or the control cells (Figure 8a). In contrast, the percentage of apoptotic cells (as measured by Annexin-V positive/PI negative staining) increases more rapidly for the anti-CD95 treated cells compared to the cross-linked anti-HLA-DR antibody fragments or the control cells (Figure 8b).

Second, we inhibited caspase activity using zDEVD-fmk, an irreversible Caspase-3 inhibitor, and zVAD-fmk, a broad spectrum Caspase inhibitor (both obtained from BioRad, Munich, DE). The mechanism of apoptosis is characterized by activity of caspases, and we hypothesized that if caspases were not necessary for anti HLA-DR mediated cell death, we would observe no change in the viability of cells undergoing cell death in the presence of these caspase inhibitors compared to those without. $2x10^5$ Priess cells were preincubated for 3 h at 37°C under 6% CO_2 with serial dilutions of the two caspase inhibitors ranging from 180 µM to 10 mM in RPMI 1640 (PAA, DE) supplemented with 2,5% heat inactivated FCS (Biowhittaker Europe, BE), 2mM L-glutamine, 1% non-essential amino acids, 1mM sodium pyruvate and 0,1mg/ml kanamycin. HLA-DR mediated cell death was induced by adding 200 nM of the human anti-HLA-DR antibody fragment MS-GPC-8 and 100 nM of the cross-linking anti-M2 mAb. An anti-CD95 induced apoptotic cell culture served as a control for the activity of inhibitors (Drenou et al., 1999). After further incubation at 37°C and 6% CO_2, cell viability after 4 and 24 h was determined by trypan blue staining and subsequent counting of non-stained cells. As we expected, cell viability of the anti-HLA-DR treated cell culture was not significantly modified by the presence of the Caspase inhibitors, while cell death induced through anti-CD95 treatment was

significantly decreased for the cell culture pre-incubated with the Caspase inhibitors. This observation supports our hypothesis that HLA-DR mediated cell death proceeds through a non-apoptotic mechanism that is independent of caspase proteases that can be inhibited by zDEVD-fm or zVAD-fmk.

16. In vivo *therapy for cancer using an HLA-DR specific antibody*

We demonstrate that antigen-binding domains of human composition can successfully be used as a therapeutic for the treatment of cancer. Immunocompromised mice - such as scid, nude or Rag-1 knockout - are inoculated with a DR+ human lymphoma or leukemia cell line of interest. The tumor cell dose, usually $1x10^6$ to $1x10^7$/mouse, is established for each tumor tested and administered subcutaneously (s.c.) or intravenously (i.v.). The mice are treated i.v. or s.c with the IgG form of the anti-HLA-DR antibody fragments MS-GPC-8, MS-GPC-8-10-57, MS-GPC-8-27-41 or others of the invention prepared as described above, using doses of 1 to 25 mg/kg over 5 days. Survival of anti-HLA-DR treated and control untreated mice is monitored for up to 8 weeks after cessation of treatment. Tumor progression in the mice inoculated s.c. is additionally quantified by measuring tumor surface area. Significant prolongation of survival of up to 80% of anti-HLA-DR treated mice is observed during the experiment, and up to 50% mice survive at the end of the experiment. In s.c. inoculated and untreated mice, the tumor reaches a surface area of 2 - 3 cm^2, while in anti-HLA-DR treated animals the tumor surface area is significantly less.

17. Immunosuppression using anti-HLA-DR antibody fragments measured by reduction in IL-2 secretion

We were surprised to observe that certain anti-HLA DR antibody fragments of the invention displayed substantial immunomodulatory properties within an assay measuring IL-2 secretion from immortalized T-cells. IgG forms of the antibody fragments MS-GPC-8-6-13, MS-GPC-8-10-57 & MS-GPC-8-27-41 showed very strong immunosuppressive properties in this assay with sub-nanomolar IC50 values and virtually 100% maximal inhibition (Figure 9a). Particularly surprising was our observation that certain monvalent compositions of the antibody fragments of the invention were able to strongly inhibit IL-2 secretion in the same assay. For example, Fab forms of the VHCDR3-selected and VLCDR3/VLCDR1 optimised antibody

fragments showed low single-digit nano-M IC50s and also almost 100% maximal inhibition (Figure 9b). Other monvalent anti-HLA DR antibody fragments of the invention showed significant immunosuppressive properties in the assay compared to control IgG and Fab fragments (Table 7).

The immunomodulatory properties of anti-HLA DR antibody fragments was investigated by measuring IL-2 secretion from the hybridoma cell line T-Hyb 1 stimulated using DR-transgenic antigen presenting cells (APC) under conditions of half-maximal antigen stimulation. IL-2 secretion was detected and measured using a standard ELISA method provided by the OptiEIA mouse IL-2 kit of Pharmingen (Torrey Pine, CA, USA). APCs were isolated from the spleen of unimmunized chimeric 0401-IE transgenic mice (Ito et al. 1996) according to standard procedures. $1.5x10^5$ APCs were added to 0.2 ml wells of 96-well in RPMI medium containing the following additives (all from Gibco BRL and PAA): 10 % FCS, 2mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate and 0.1 g/l kanamycin. Hen egg ovalbumin was added to a final concentration of 200 µg/ml in a final volume of 100 ul of the above medium, the cells incubated with this antigen for 30 min at 37°C under 6% CO_2. Anti-HLA DR antibody fragments were added to each well at various concentrations (typically in a range from 0.1 to 200 nM), the plate incubated for 1 h at 37°C/6% CO_2 and $2x10^5$ T-Hyb 1 cells added to give a final volume of 200 µl in the above medium. After incubation for 24 h, 100 µl of supernatant was transferred to an ELISA plate (Nunc-Immuno Plate MaxiSorp surface, Nunc, Roskilde, DK) previously coated with IL-2 Capture Antibody (BD Pharmingen, Torrey Pine, CA, USA), the amount of IL-2 was quantified according to the manufacturer's directions using the OptiEIA Mouse IL-2 kit and the plate read using a Victor V reader (Wallac, Finland). Secreted IL-2 in pg/ml was calibrated using the IL-2 standards provided in the kit.

The T-cell hybridoma line T-Hyb1 was established by fusion of a T-cell receptor negative variant of the thymoma line BW 5147 (ATCC) and lymph node cells from chimeric 0401-IE transgenic mice previously immunized with hen egg ovalbumin (Ito et al. 1996). The clone T-Hyb1 was selected for the assay since it responded to antigen specific stimulation with high IL-2 secretion.

18. Immunosuppression using an HLA-DR specific antibody measured by T cell proliferation

Immunomodulatory properties of the anti-HLA DR antibody fragments were also seen within an assay that measures T cell proliferation. The IC50 value for inhibition of T cell proliferation of the IgG form of MS-GPC-8-10-57 and MS-GPC-8-27-41 were 11 and 20 nM respectively (Figure 10). The anti-HLA DR antibody fragments were tested as follows to inhibit the proliferative T cell response of antigen-primed lymph node cells from mice carrying a chimeric mouse-human class II transgene with an RA-associated peptide binding site, and lack murine class II molecules (Muller et al., 1990; Woods et al., 1994; Current Protocols in Immunology, Vol. 2, 7.21; Ito et al., 1996). Here, the immunization takes place *in vivo*, but the inhibition and readout are *ex vivo*. Transgenic mice expressing MHC class II molecules with binding sites of the RA associated molecule, DRB*0401 were commercially obtained. These mice lack murine MHC class II, and thus, all Th responses are channelled through a single human RA-associated MHC class II molecule (Ito et al. 1996). These transgenic mice represent a model for testing human class II antagonists.

The inhibitory effect of the anti-HLA-DR antibody fragments and their IgG forms were tested on T-cell proliferation measured using chimeric T-cells and antigen presenting cells isolated from the lymph nodes of chimeric 0401-IE transgenic mice (Taconic, USA) previously immunized with hen egg ovalbumin (Ito et al. 1996) according to standard procedures. 1.5×10^5 cells are incubated in 0.2 ml wells of 96-well tissue culture plates in the presence of ovalbumin (30 µg per well - half-maximal stimulatory concentration) and a dilution series of the anti-HLA DR antibody fragment or IgG form under test (0.1 nM - 200 nM) in serum free HL-1 medium containing 2 mM L-glutamine and 0.1 g/l Kanamycin for three days. Antigen specific proliferation is measured by ^{3}H-methyl-thymidin(1 µCi/well) incorporation during the last 16h of culture (Falcioni et al., 1999). Cells are harvested, and ^{3}H incorporation measured using a scintillation counter (TopCount, Wallac Finland). Inhibition of T-cell proliferation on treatment with the anti-HLA DR antibody fragment and its IgG form may be observed by comparison to control wells containing antigen.

19. Selection of useful polypeptide for the treatment of cancers

In order to select the most appropriate protein/peptide to enter further experiments and to assess its suitability for use in a therapeutic composition for the treatment of cancers, additional data are collected. Such data for each IgG form of the anti-HLA antigen antibody fragments can include the binding affinity, *in vitro* killing efficiency as measured by EC50 and cytotoxicity across a panel of tumor cell lines, the maximal percentage cell killing as estimated *in vitro*, and tumor reduction data and mouse survival data from *in vivo* animal models.

The IgG form of the anti-HLA antigen antibody fragments that shows the highest affinity, the lowest EC50 for killing, the highest maximal percentage cell killing and broadest across various tumor cell lines, the best tumor reduction data and/or the best mouse-survival data may be chosen to enter further experiments. Such experiments may include, for example, therapeutic profiling and toxicology in animals and phase I clinical trials in humans.

20. Selection of useful polypeptide for the treatment of diseases of the immune system

In order to select the most appropriate protein/peptide to enter further experiments and to assess its suitability for use in a therapeutic composition for the treatment of diseases of the immune system, additional data are collected. Such data for each monovalent antibody fragment or IgG form of the anti-HLA antigen antibody fragments can include the affinity, reactivity, specificity, IC50-values, for inhibition of IL-2 secretion and of T-cell proliferation, or *in vitro* killing efficiency as measured by EC50 and the maximal percentage cell killing as estimated *in vitro*, and DR-transgenic models of transplant rejection and graft vs. host disease.

The antibody fragment or IgG form of the anti-HLA antigen antibody fragments that shows the lowest EC50, highest affinity, highest killing, best specificity and/or greatest inhibition of T-cell proliferation or IL-2 secretion, and high efficacy in inhibiting transplant rejection and/or graft vs. host disease in appropriate models, might be chosen to enter further experiments. Such experiments may include, for example, therapeutic profiling and toxicology in animals and phase I clinical trials in humans.

Table 1:

VH and VL families, VL CDR1 and VH/VL CDR 3 sequences of HLA-DR-specific polypeptides

Clone	VH	CDR3 Length	**VH-CDR3-Seq.**	VL	**VL-CDR1-Seq.**	CDR3 Length	**VL-CDR3-Seq.**	Families
MS-GPC-1	**H2**	**10**	**QYGHRGGFDH**	**λ 1**	SGSSSNIGSNYVS	**8**	**QSYDFNES**	**H2 λ 1**
MS-GPC-6	**H3**	**9**	**GYGRYSPDL**	**K3**	RASQSVSSSYLA	**8**	**QQYSNLPF**	**H3 K 3**
MS-GPC-8	**H2**	**10**	**SPRYRGAFDY**	**λ 1**	SGSSSNIGSNYVS	**8**	**QSYDMPQA**	**H2 λ 1**
MS-GPC-10	**H2**	**10**	**QLHYRGGFDL**	**λ 1**	SGSSSNIGSNYVS	**8**	**QSYDLTMG**	**H2 λ 1**
MS-GPC-8-1	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDFSHY**	H2 λ 1
MS-GPC-8-6	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDYDHY**	H2 λ 1
MS-GPC-8-9	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDIQLH**	H2 λ 1
MS-GPC-8-10	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDLIRH**	H2 λ 1
MS-GPC-8-17	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDFSVY**	H2 λ 1
MS-GPC-8-18	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDFSIY**	H2 λ 1
MS-GPC-8-27	H2	10	SPRYRGAFDY	λ 1	SGSSSNIGSNYVS	8	**QSYDMNVH**	H2 λ 1

MS-GPC-8-6-2	H2	10	SPRYRGAFDY	λ 1	SGSESNIGSNYVH	8	QSYDYDHY	H2 λ 1
MS-GPC-8-6-19	H2	10	SPRYRGAFDY	λ 1	SGSESNIGSNYVA	8	QSYDYDHY	H2 λ 1
MS-GPC-8-6-27	H2	10	SPRYRGAFDY	λ 1	SGSDSNIGANYVT	8	QSYDYDHY	H2 λ 1
MS-GPC-8-6-45	H2	10	SPRYRGAFDY	λ 1	SGSEPNIGSNYVF	8	QSYDYDHY	H2 λ 1
MS-GPC-8-6-13	H2	10	SPRYRGAFDY	λ 1	SGSESNIGANYVT	8	QSYDYDHY	H2 λ 1
MS-GPC-8-6-47	H2	10	SPRYRGAFDY	λ 1	SGSESNIGSNYVS	8	QSYDYDHY	H2 λ 1
MS-GPC-8-10-57	H2	10	SPRYRGAFDY	λ 1	SGSESNIGNNYVQ	8	QSYDLIRH	H2 λ 1
MS-GPC-8-27-7	H2	10	SPRYRGAFDY	λ 1	SGSESNIGNNYVG	8	QSYDMNVH	H2 λ 1
MS-GPC-8-27-10	H2	10	SPRYRGAFDY	λ 1	SGSESNIGANYVN	8	QSYDMNVH	H2 λ 1
MS-GPC-8-27-41	H2	10	SPRYRGAFDY	λ 1	SGSESNIGNNYVQ	8	QSYDMNVH	H2 λ 1

Table 2:

Steps in Antibody optimisation	Fab	k_{on} [$s^{-1}M^{-1}$] x 10^5 +/- SD	k_{off} [s^{-1}] x 10^{-3} +/- SD	K_D [nM] +/- SD	L-CDR3	L-CDR1
Parental Fab	MS-GPC-8	0.99 ± 0.40	29.0 ± 8.40	346.1 ± 140.5[a)]	QSYDMPQA	SGSSSNIGSNYVS
L-CDR3-optim.	-8-1	1.93	20.9	108[e)]		
L-CDR3-optim.	-8-6	0.96 ± 0.14	5.48 ± 0.73	58.6 ± 11.7[b)]		
L-CDR3-optim.	-8-9	1.85	16.6	90.1[e)]		
L-CDR3-optim.	-8-10	nd	7.0[e)]	nd		
L-CDR3-optim.	-8-17	1.0	5.48	54.7[e)]		
L-CDR3-optim.	-8-18	1.06	8.3	78.3[e)]		
L-CDR3-optim.	-8-27	nd	6.6[e)]	nd		
L-CDR3-optim.	-8-6	0.96 ± 0.14	5.48 ± 0.73	58.6 ± 11.7[b)]	QSYDYDHY	SGSSSNIGSNYVS
L-CDR3+1-opt.	-8-6-2	1.23 ± 0.11	0.94 ± 0.07	7.61 ± 0.25[c)]	QSYDYDHY	SGSESNIGSNYVH
L-CDR3+1-opt.	-8-6-19	1.10 ± 0.08	0.96 ± 0.15	8.74 ± 1.33[c)]	QSYDYDHY	SGSESNIGSNYVA
L-CDR3+1-opt.	-8-6-27	1.80 ± 0.24	1.10 ± 0.15	6.30 ± 0.63[d)]	QSYDYDHY	SGSDSNIGANYVT
L-CDR3+1-opt.	-8-6-45	1.20 ± 0.07	1.03 ± 0.04	8.63 ± 0.61[c)]	QSYDYDHY	SGSEPNIGSNYVF
L-CDR3+1-opt.	-8-6-13	1.90 ± 0.26	0.55 ± 0.05	2.96 ± 0.46[c)]	QSYDYDHY	SGSESNIGANYVT
L-CDR3+1-opt.	-8-6-47	1.97 ± 0.29	0.62 ± 0.04	3.18 ± 0.33[c)]	QSYDYDHY	SGSESNIGSNYVS
L-CDR3+1-opt.	-8-10-57	1.65 ± 0.21	0.44 ± 0.06	2.67 ± 0.25[c)]	QSYDLIRH	SGSESNIGNNYVQ
L-CDR3+1-opt.	-8-27-7	1.74 ± 0.21	0.57 ± 0.07	3.30 ± 0.34[d)]	QSYDMNVH	SGSESNIGNNYVG
L-CDR3+1-opt.	-8-27-10	1.76 ± 0.21	0.53 ± 0.05	3.01 ± 0.21[c)]	QSYDMNVH	SGSESNIGANYVN

L-CDR3+1-opt.	-8-27-41	1.67 ± 0.16	0.49 ± 0.03	2.93 ± 0.27[d)]	QSYDMNVH	SGSESNIGNNYVQ

a) Affinity data of MS-GPC-8 are based on 8 different Fab-preparations which were measured on 4 different chips (2 x 500, 1000, 4000RU)

b) For MS-GPC-8-6 mean and standard deviation of 3 different preparations on 3 different chips (500, 4000, 3000RU) is shown.

c) 3000RU MHCII were immobilized on a CM5-chip. For each measurement 7 different concentrations from 1µM to 16nM were injected on the surface. Dissociation time: 150sec, regeneration was reached by 6µl 10mM Glycine pH2.3 followed by 8µl 7.5mM NaOH. For MS-GPC-8-6-19 mean and standard deviation of 4 different preparations are shown whereas for all other binders mean and standard deviation of 3 different preparations are shown.

d) One protein preparation is measured on 3 different chips (3000, 2800 and 6500RU).

e) Affinity determination of maturated MHCII binder on a 4000RU density chips; single measurement.

Molecular weights were determined after size exclusion chromatography and found 100% monomeric with the right molecular weight between 45 and 48 kDa.

Table 3a

Affinities of selected IgG4 monoclonal antibodies constructed from F_{ab}'s. Errors represent standard deviations

Binder (IgG_4)	k_{on} [M^{-1} s^{-1}] x10^5	k_{off} [s^{-1}] x10^{-5}	K_D [nM]
MS-GPC-8-27-41	1.1 ± 0.2	3,1 ± 0.4	0,31 ± 0.06
MS-GPC-8-6-13	0,7 ± 0.1	3 ± 1	0,5 ± 0.2
MS-GPC-8-10-57	0,7 ± 0.2	4 ± 1	0,6 ± 0.2

Table 3b

Affinities of binders obtained out of affinity maturation of CDR1 light chain optimisation following CDR3 heavy chain optimisation. Errors represent standard deviations

Binder (F_{ab})	k_{on} [$M^{-1}s^{-1}$] x10^5	k_{off} [s^{-1}] x10^{-3}	K_D [nM]
MS-GPC-8-6-2	1.2 ± 0.1	0.94 ± 0.07	7.6 ± 0.3
MS-GPC-8-6-19	1.1 ± 0.1	1.0 ± 0.2	9 ± 1
MS-GPC-8-6-27	1.8 ± 0.2	1.1 ± 0.2	6.3 ± 0.6
MS-GPC-8-6-45	1.20 ± 0.07	1.03 ± 0.04	8.6 ± 0.6
MS-GPC-8-6-13	1.9 ± 0.3	0.55 ± 0.05	3.0 ± 0.5
MS-GPC-8-6-47	2.0 ± 0.3	0.62 ± 0.04	3.2 ± 0.3
MS-GPC-8-10-57	1.7 ± 0.2	0.44 ± 0.06	2.7 ± 0.3
MS-GPC-8-27-7	1.7 ± 0.2	0.57 ± 0.07	3.3 ± 0.3
MS-GPC-8-27-10	1.8 ± 0.2	0.53 ± 0.05	3.0 ± 0.2
MS-GPC-8-27-41	1.7 ± 0.2	0.49 ± 0.03	2.9 ± 0.3

Table 3c

Binders obtained out of affinity maturation of GPC8 by CDR3 light chain optimisation

Binder (F_{ab})	k_{on} [$M^{-1}s^{-1}$] x10^5	k_{off} [s^{-1}] x10^{-3}	K_D [nM]
MS-GPC 8-18	1.06	8.3	78.3
MS-GPC 8-9	1.85	16.6	90.1
MS-GPC 8-1	1.93	20.9	108
MS-GPC 8-17	1.0	5.48	54.7
MS-GPC-8-6[a)]	1.2 +/- 0.1	5.5 +/- 0.7	8 +/- 12

Chip density 4000RU MHCII

a) For MS-GPC-8-6 mean and standard deviation of 3 different preparations on 3 different chips (500, 4000, 3000RU) is shown.

Table 3d

Binders obtained out of HuCAL in scFv form and their converted Fabs

Binder	scF_V			F_{ab}		
	k_{on} [$M^{-1}s^{-1}$] x10^5	k_{off} [s^{-1}] x10^{-3}	K_D [nM]	k_{on} [$M^{-1}s^{-1}$] x10^5	k_{off} [s^{-1}] x10^{-3}	K_D [nM]
MS-GPC 1	0.413	61	1500	0.639	53	820
MS-GPC 6	0.435	200	4600	0.135	114	8470 (1 curve)
MS-GPC 8	0.114	76	560	0.99 +/- 0.40	29.0 +/- 8.4	346[a)] +/- 141
MS-GPC 10	0.187	180	9625	0.22	63	2860

Chip density 500RU MHCII

a) Affinity data of MS-GPC-8 are based on 8 different Fab-preparations which were measured on 4 different chips (2 x 500, 1000, 4000RU) and are shown with standard deviation.

Table 4

Killing efficiency after 4 hour incubation of cells with cross-linked anti-HLA-DR antibody fragments, and maximum killing after 24 hour incubation

Cross-linked Fab fragment	Killing efficiency against GRANTA	Maximum killing against Priess
MS-GPC-1	+	+
MS-GPC-6	+	+
MS-GPC-8	+	+
MS-GPC-10	+	+
MS-GPC-8-6	++	++
MS-GPC-8-17	++	++
MS-GPC-8-6-13	+++	+++
MS-GPC-8-10-57	+++	+++
MS-GPC-8-27-41	+++	+++

Table 5

Killing efficiency of anti-HLA-DR IgG antibodies of human composition compared to murine anti-HLA-DR antibodies against a panel of lymphoid tumor cell lines.

Cell line			HLA-DR expression mean-FL	% Killing by mAb					
				murine mAbs		human mAbs			
Name	DR type	Type	L243	L243	8D1	MS-GPC-8	8-27-41	8-10-57	8-6-13
LG-2	1,1	B-lymphoblastoid	458	79	85	86	87	88	82
Priess	4,4	B-lymphoblastoid	621	87	83	85	88	93	74
ARH-77	12	B-lymphoblastoid	301	88	73	84	85	88	87
GRANTA-519	2,11	B cell non-Hodgkin	1465	83	56	76	78	78	73
KARPAS-422	2,4	B cell non-Hodgkin	186	25	32	51	66	68	71
KARPAS-299	1,2	T cell non-Hodgkin	919	78	25	81	82	79	76
DOHH-2	1,2	B cell lymphoma	444	29	23	58	59	60	53
SR-786	1,2	T cell lymphoma	142	3	8	1	53	44	26
MHH-CALL-4	1,2	B-ALL	348	35	41	43	63	46	43
MN-60	10,13	B-ALL	1120	46	22	71	69	66	67
BJAB	12,13	Burkitt lymph.	338	53	59	49	71	67	64
RAJI	10, 17	Burkitt lymph.	617	69	64	81	84	86	83
L-428	12	Hodgkin's lymph.	244	82	81	82	91	91	92

HDLM-2		Hodgkin's lymph.	326	77	73	89	88	84	90
HD-MY-Z		Hodgkin's lymph.	79	35	39	49	69	57	72
KM-H2		Hodgkin's lymph.	619	81	56	75	86	88	87
L1236		Hodgkin's lymph.	41	52	62	44	63	66	66
BONNA-12		hairy cell leuk.	2431	92	91	91	92	91	86
HC-1		hairy cell leuk.	372	88	89	89	93	86	93
NALM-1	1,4	CML	1078	44	4	83	82	78	65
L-363		plasma cell leu.	49	6	5	26	26	24	19
EOL-1		AML (eosinophil)	536	22	13	36	69	49	53
LP-1		multiple myeloma	315	12	0	61	73	70	73
RPMI-8226		multiple myeloma	19	6	0	14	29	26	19
MHH-PREB-1		B cell non-Hodgkin	175	3	3	2	4	8	11
MHH-CALL-2		B cell precursor leu.	+	5	5				
OPM-2		multiple myeloma	3	13	0	8	1	4	5
KASUMI-1		AML	5	0	0	8	10	10	6
HL-60		AML	3	18	0	3	15	9	22
LAMA-84		CML	7	7	9	5	11	5	7

% Killing: 100 - % viable cells after a 4h treatment with 200 nM murine or 50 nM human mAb at 37°C.

Table 6

EC50 values for certain anti-HLA-DR antibody fragments of the invention in a cell-killing assay against lymphoid tumor cells. All EC50 refer to nanomolar concentrations of the bivalent agent (IgG or cross-linked Fab) such that values for cross-linked Fab and IgG forms can be compared.

Antibody fragment	Form	Cell line tested	EC50 of cell killing (nM) +/- SE for bivalent agent
MS-GPC-1	Fab	PRIESS	54 ± 14
MS-GPC-8	Fab	PRIESS	31 ± 9
MS-GPC-10	Fab	PRIESS	33 ± 5
MS-GPC-8-17	Fab	PRIESS	16 ± 4
MS-GPC-8-6-2	Fab	PRIESS	8 ± 2
MS-GPC-8-10-57	Fab	LG2	7.2
MS-GPC-8-27-41	Fab	LG2	7.2
MS-GPC-8-27-41	Fab	PRIESS	7.7
MS-GPC-8	IgG4	PRIESS	8.3
MS-GPC-8-27-41	IgG4	PRIESS	1.1 ± 0.1
MS-GPC-8-10-57	IgG4	PRIESS	1.1 ± 0.2
MS-GPC-8-27-41	IgG4	LG2	1.23 ± 0.2
MS-GPC-8-10-57	IgG4	LG2	1.0 ± 0.1
8D1	mIgG	PRIESS	33
L243	mIgG	PRIESS	47

Table 7

IC50 values for certain anti-HLA-DR antibody fragments of the invention in an assay to determine IL-2 secretion after antigen-specific stimulation of T-Hyb 1 cells. IC50 for the IgG forms (bivalent) are represented as molar concentrations, while in order to provide easy comparison, IC50s for the Fab forms (monovalent) are expressed in terms of half the concentration of the Fab to enable direct comparison to IgG forms.

Anti-HLA-DR antibody fragment	Form	IC50 (IgG/nM) ((Fab)/2/nM)		Maximum inhibition(%)
		Mean	SE	
MS-GPC-8-10-57	IgG	0.31	0.01	100
MS-GPC-8-27-41	IgG	0.28	0.07	100
MS-GPC-8-6-13	IgG	0.42	0.06	100
MS-GPC-8-6-2	IgG	3.6	1.1	100
MS-GPC-8-6	IgG	6.7	2.0	100
MS-GPC-8	IgG	11.0	0.8	100
MS-GPC-8-6-2	Fab	4.7	1.9	100
MS-GPC-8-6-13	Fab	2.1	0.8	100
MS-GPC-8-6-19	Fab	5.3	0.2	100
MS-GPC-8-10-57	Fab	2.9	1.0	100
MS-GPC-8-6-27	Fab	3.0	1.2	100
MS-GPC-8-6-47	Fab	2.6	0.6	100
MS-GPC-8-27-7	Fab	5.9	2.2	100
MS-GPC-8-27-10	Fab	7.3	1.9	100
MS-GPC-8-27-41	Fab	3.6	0.7	100
MS-GPC-8-6	Fab	20		100
MS-GPC-8	Fab	110		100

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Claims

1. A composition including a polypeptide comprising an antibody-based antigen-binding domain of human composition with binding specificity for an antigen expressed on the surface of a human cell, wherein treating cells expressing said antigen with a multivalent polypeptide having two or more of said antigen-binding domains causes or leads to killing of said cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said killing.

2. A composition including a polypeptide comprising an antibody-based antigen-binding domain which binds to human HLA DR with a K_d of 1μM or less, wherein treating cells expressing HLA DR with a multivalent polypeptide having two or more of said antigen-binding domains causes or leads to killing of said cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said killing.

3. A composition including a multivalent polypeptide comprising a plurality of antibody-based antigen-binding domains of human composition which specifically bind to human HLA DR, wherein treating cells expressing HLA DR with said multivalent polypeptide causes or leads to killing of said cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said killing, wherein said antigen-binding domains individually bind to human HLA DR with a K_d of 1μM or less.

4. A composition including a multivalent polypeptide comprising a plurality of antibody-based antigen-binding domains of human composition which specifically bind to human HLA DR, wherein treating cells expressing HLA DR with said multivalent polypeptide causes or leads to killing of said cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said cell killing, wherein said multivalent polypeptide has an EC_{50} of 100 nM or less for killing activated lymphoid cells.

5. A composition including a polypeptide comprising at least one antibody-based antigen-binding domain that binds to human HLA DR with a K_d of 1μM or less, said antigen-binding domain being isolated by a method which includes isolation of VL and VH domains of human composition from a recombinant antibody library by ability to bind to at least one epitope of human HLA DR, wherein treating cells expressing HLA DR with a multivalent polypeptide having two or more of said antigen binding domains causes or leads to killing of said cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said killing.

6. The composition of claim 5, wherein the method for isolating the antigen-binding domain includes the further steps of:

   a. generating a library of variants of at least one of the CDR1, CDR2 and CDR3 sequences of one or both of the VL and VH domains, and

   b. isolation of VL and VH domains from the library of variants by ability to bind to human HLA DR with a K_d of 1μM or less.

7. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} for killing transformed cells at least 5-fold lower than the EC_{50} for killing normal cells.

8. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} for killing activated cells at least 5-fold lower than the EC_{50} for killing unactivated cells.

9. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} of 50nM or less for killing transformed cells.

10. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} for killing lymphoid tumor cells of 10nM or less.

11. The composition of any of claim 1-6 or 8, wherein the multivalent polypeptide kills activated lymphoid cells.

12. The composition of claim 11, wherein said activated lymphoid cells are lymphoid tumor cells representing a disease selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia, T cell lymphoma, T cell non-Hodgkin lymphoma, chronic myeloid leukemia, chronic lymphoid leukemia, and multiple myeloid leukemia.

13. The composition of claim 11, wherein said activated lymphoid cells are from a cell line taken from the list of Priess, GRANTA-519, KARPAS-422, KARPAS-299, DOHH-2, SR-786, MHH-CALL-4, MN-60, BJAB, RAJI, L-428, HDLM-2, HD-MY-Z, KM-H2, L1236, BONNA-12, HC-1, NALM-1, L-363, EOL-1, LP-1, RPMI-8226, and MHH-PREB-1 cell lines.

14. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} of 100nM or less for killing cells of at least one of lymphoid tumor cell lines selected from the list of KARPAS-422, DOHH-2, SR-7, MHH-CALL-4, MN-60, HD-MY-Z, NALM-1 and LP-1.

15. The composition of any of claims 1-6, wherein the multivalent polypeptide has an EC_{50} of 50nM or less for killing cells from at least one lymphoid tumor cell line selected from the list of KARPAS-422, DOHH-2, MN-60, NALM-1 and LP-1.

16. The composition of any of claims 1-6, wherein the multivalent polypeptide hasan EC_{50} of 10nM or less for killing cells from at least one B cell lymphoblastoid cell line selected from the list LG2 and Priess.

17. The composition of any of claims 1-6, wherein said cells are non-lymphoid cells that express MHC class II molecules

18. The composition of any of claims 1-6, wherein said antigen-binding domain binds to the β-chain of HLA-DR.

19. The composition of claim 18, wherein said antigen-binding domain binds to the first domain of the β-chain of HLA-DR.

20. The composition of any of claims 1-6, wherein said antigen-binding domain binds to one or more HLA-DR types selected from the group consisting of DR1-0101, DR2-15021, DR3-0301, DR4Dw4-0401, DR4Dw10-0402, DR4Dw14-0404, DR6-1302, DR6-1401, DR8-8031, DR9-9012, DRw53-B4*0101 and DRw52-B3*0101.

21. The composition of claim 20, wherein said antigen-binding domain binds to at least 5 different of said HLA-DR types.

22. The composition of any one of claims 1-6, wherein said antigen-binding domain includes a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

23. The composition of any one of claims 1-6, wherein said antigen-binding domain includes of a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3, VL CDR1 And VL CDR3 is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

24. The composition of any one of claims 1-23, wherein said antigen-binding domain includes a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

wherein each n independently represents any amino acid residue; and/or

wherein the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

wherein each n independently represents any amino acid residue.

25. The composition of claim 24, wherein the VH CDR3 sequence is SPRYGAFDY and/or the VL CDR3 sequence is QSYDLIRH or QSYDMNVH.

26. The composition of any one of claims 1-23, wherein said antigen-binding domain competes for antigen binding with an antibody including a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

each n independently represents any amino acid residue; and/or

the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

each n independently represents any amino acid residue.

27. The composition of claim 26, wherein the VH CDR3 sequence is SPRYGAFDY and/or the VL CDR3 sequence is QSYDLIRH or QSYDMNVH.

28. The composition of any one of claims 1-27, wherein said antigen-binding domain includes a VL CDR1 sequence represented in the general formula

SGSnnNIGnNYVn

wherein each n independently represents any amino acid residue.

29. The composition of claim 28, wherein the CDR1 sequence is SGSESNIGNNYVQ.

30. The composition of any of claims 1-29, wherein the mechanism of said killing involves an innate pre-programmed process of said cell.

31. The composition of claim 30, wherein said killing is non-apoptotic.

32. The composition of claim 30, wherein said killing is dependent on the action of non-caspase proteases, and/or wherein said killing cannot be inhibited by zVAD-fmk or zDEVD-fmk.

33. The composition of any one of claims 1-32, wherein said antibody-based antigen-binding domain is part of a multivalent polypeptide including at least a $F(ab')_2$ antibody fragment or a mini-antibody fragment.

34. The composition of any one of claims 1-32, wherein said antibody-based antigen-binding domain is part of a multivalent polypeptide comprising at least two monovalent antibody fragments selected from Fv, scFv, dsFv and Fab fragments, and further comprises a cross-linking moiety or moieties.

35. The composition of any one of claims 1-32, wherein said antibody-based antigen-binding domain is part of a multivalent polypeptide comprising at least one full antibody selected from the antibodies of classes IgG1, 2a, 2b, 3, 4, IgA, and IgM.

36. The composition of any one of claims 1-32, wherein said antibody-based antigen-binding domain is part of a multivalent polypeptide that is formed prior to binding to a cell.

37. The composition of any one of claims 1-32, wherein said antibody-based antigen-binding domain is part of a multivalent polypeptide that is formed after binding to a cell.

38. The composition of claim 3 or 4, wherein the antigen binding sites are cross-linked to a polymer.

39. A nucleic acid comprising a protein coding sequence for an antigen-binding domain comprised in any of claims 1-32, or a multivalent polypeptide thereof.

40. A vector comprising the nucleic acid of claim 39, and a transcriptional regulatory sequence operably linked thereto.

41. A host cell harboring at least one nucleic acid of claim 39 or the vector of claim 40.

42. A method for the production of composition comprising a multivalent polypeptide that causes or leads to killing of cells in a manner where neither cytotoxic entities nor immunological mechanisms are needed for said killing, comprising culturing the cells

of claim 41 under conditions wherein the nucleic acid is expressed either as a multivalent polypeptide or as a polypeptide comprising at least one antigen binding domains which is subsequently treated to form a multivalent polypeptide composition.

43. The composition of any of claims 1-38, formulated in a pharmaceutically acceptable carrier and/or diluent.

44. The use of a composition of any of claims 1-38, for preparing a pharmaceutical preparation for the treatment of animals.

45. The use of a nucleic acid of claim 39 for preparing a pharmaceutical preparation for the treatment of animals

46. The use of a host cell of claim 41 for preparing a pharmaceutical preparation for the treatment of animals

47. The use of the method of claim 42 for preparing a pharmaceutical preparation for the treatment of animals

48. The use according to claim 44-47, wherein said animal is a human.

49. The use according to claim 44-48, for the treatment of cell proliferative disorders, wherein said antibody-based antigen binding domain is part of a multivalent polypeptide.

50. The use according to claim 49, wherein said treatment is the treatment of disorders involving transformed cells expressing MHC class II antigens.

51. The use according claim 49 or 50, wherein said treatment is the treatment of a disorder selected from B cell non-Hodgkin lymphoma, B cell lymphoma, B cell acute lymphoid leukemia, Burkitt lymphoma, Hodgkin lymphoma, hairy cell leukemia, acute myeloid leukemia, T cell lymphoma, T cell non-Hodgkin lymphoma, chronic myeloid leukemia, chronic lymphoid leukemia, and multiple myeloid leukemia.

52. The use according to any of claims 44-48, wherein said treatment is the treatment of disorders involving unwanted activation of cells of the immune system, such as lymphoid cells expressing MHC class II.

53. The use according to any of claims 44-48, wherein said treatment is the treatment of a disorder selected from rheumatoid arthritis, juvenile arthritis, multiple sclerosis, Grave's disease, insulin-dependent diabetes, narcolepsy, psoriasis, systemic lupus erythematosus, ankylosing spondylitis, transplant rejection, graft vs. host disease, Hashimoto's disease, myasthenia gravis, pemphigus vulgaris, glomerulonephritis, thyroiditis, pancreatitis, insulitis, primary biliary cirrhosis, irritable bowel disease and Sjogren syndrome.

54. The use according to any of claims 44-48, wherein said disorder is selected from myasthenia gravis, rheumatoid arthritis, multiple sclerosis, transplant rejection and graft vs. host disease.

55. A diagnostic composition including the composition of any of claims 1-38.

56. A diagnostic composition including the composition of any of claims 1-38 and a cross-linking moiety or moieties.

57. A method for killing a cell expressing an antigen on the surface of said cell comprising the step of treating the cell with a plurality of antigen-binding domains of any one of claims 1-38, wherein said antibody-based antigen-binding domains are part of a multivalent polypeptide, and where neither cytotoxic entities nor immunological mechanisms are needed to causes or leads to said killing

58. A method to identify patients that can be treated with a composition of any of claims 1-38, formulated in a pharmaceutically acceptable carrier and/or diluent comprising the steps of

    a. Isolating cells from a patient;

    b. Contacting said cells with the composition of any of claims 1-38; and

    c. Measuring the degree of killing or immunosuppression of said cells.

59. A kit to identify patients that can be treated with a composition of any of claims 1-38, formulated in a pharmaceutically acceptable carrier and/or diluent comprising

    a. A composition of any of claims 1-38; and

    b. Means to measure the degree of killing or immunosuppression of said cells.

60. A kit comprising

    a. a composition according to any one of claims 1-38, and

    b. a cross-linking moiety.

61. A kit comprising

    a. a composition according to any one of claims 1-38, and

    b. a detectable moiety or moieties, and

    c. reagents and/or solutions to effect and/or detect binding of (i) to an antigen.

62. A cytotoxic composition comprising a composition of any one of claims 1-38 operably linked to a cytotoxic agent.

63. An immunogenic composition comprising a composition of any one of claims 1-38 operably linked to an immunogenic agent.

64. A method to kill a cell comprising contacting said cell with a composition of any one of claims 1-38 operably linked a cytotoxic or immunogenic agent.

65. The use of a composition of any one of claims 1-38 operable linked a cytotoxic or immunogenic agent for preparing a pharmaceutical preparation for the treatment of animals.

66. A composition including a polypeptide comprising at least one antibody-based antigen-binding domain with a binding specificity for a human MHC class II antigen with a K_d of 1µM or less, wherein treating cells expressing said antigen with said polypeptide causes or leads to suppression of an immune response.

67. A composition including a polypeptide comprising at least one antibody-based antigen-binding domain with a binding specificity for human HLA DR antigen, wherein treating cells expressing HLA DR with said polypeptide causes or leads to suppression of an immune response, and wherein said antigen-binding domain includes a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

68. A composition including a polypeptide comprising at least one antibody-based antigen-binding domain with a binding specificity for a human MHC class II antigen with a K_d of 1µM or less, said antigen-binding domain being isolated by a method which includes isolation of VL and VH domains of human composition from a recombinant antibody library by ability to bind to human MHC class II antigen, wherein treating cells expressing MHC Class II with said polypeptide causes or leads to suppression of an immune response.

69. The composition of claim 68, wherein the method for isolating the antigen-binding domain includes the further steps of:

    a. generating a library of variants at least one of the CDR1, CDR2 and CDR3 sequences of one or both of the VL and VH domains, and

    b. isolation of VL and VH domains from the library of variants by ability to bind to human MHC class II antigen with a K_d of 1µM or less;

c. (optionally) repeating steps (a) and (b) with at least one other of the CDR1, CDR2 and CDR3 sequences.

70. The composition of any of claims 67, 68 or 69, wherein said antigen-binding domain binds to HLA-DR

71. The composition of any of claims 66 or 70 wherein said antigen-binding domain binds to the β-chain of HLA-DR.

72. The composition of claim 71, wherein said antigen-binding domain binds to an epitope of the first domain of the β-chain of HLA-DR.

73. The composition of any of claims 66-72, wherein said cells are lymphoids cells.

74. The composition of any of claims 66-72, wherein said cells are non-lymphoid cells and express MHC class II antigens.

75. The composition of any of claims 66-74, having an IC_{50} for suppressing an immune response of 1 μ M or less.

76. The composition of any of claims 66-74, having an IC50 for inhibition of IL-2 secretion of 1 μ M or less

77. The composition of any of claims 66-74, having an IC50 for inhibiting T cell proliferation of 1 μ M or less

78. The composition of any of claims 66-77, wherein said antigen-binding domain binds to one or more HLA-DR types selected from the group consisting of DR1-0101, DR2-15021, DR3-0301, DR4Dw4-0401, DR4Dw10-0402, DR4Dw14-0404, DR6-1302, DR6-1401, DR8-8031, DR9-9012, DRw53-B4*0101 and DRw52-B3*0101.

79. The composition of claim 78, wherein said antigen-binding domain binds to at least 5 different of said HLA-DR types.

80. The composition of any of claims 66-79, wherein said antigen-binding domain includes a combination of a VH domain and a VL domain, wherein said combination is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-6, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

81. The composition of any one of claims 66-77, wherein said antigen-binding domain includes of a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3, VL CDR1 And VL CDR3 is found in one of the clones taken from the list of MS-GPC-1, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

82. The composition of any of claims 66-77, wherein said antigen-binding domain includes a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

wherein each n independently represents any amino acid residue; and/or

wherein the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

wherein each n independently represents any amino acid residue.

83. The composition of claim 82, wherein the VH CDR3 sequence is SPRYGAFDY and/or the VL CDR3 sequence is QSYDLIRH or QSYDMNVH.

84. The composition of any of claims 66-77, wherein said antigen-binding domain competes for antigen binding with an antibody including a combination of HuCAL VH2 and HuCAL Vλ1, wherein the VH CDR3 sequence is taken from the consensus CDR3 sequence

nnnnRGnFDn

each n independently represents any amino acid residue; and/or

the VL CDR3 sequence is taken from the consensus CDR3 sequence

QSYDnnnn

each n independently represents any amino acid residue.

85. The composition of claim 84, wherein the VH CDR3 sequence is SPRYGAFDY and/or the VL CDR3 sequence is QSYDLIRH or QSYDMNVH.

86. The composition of any of claims 66-85, wherein said antigen-binding domain includes a VL CDR1 sequence represented in the general formula

SGSnnNIGnNYVn

wherein each n independently represents any amino acid residue.

87. The composition of claim 86, wherein the CDR1 sequence is SGSESNIGNNYVQ.

88. The composition of any one of claims 66-85, wherein said suppression of an immune response is brought about by or manifests itself in down-regulation of expression of said antigen expressed on the surface of said cell.

89. The composition of any one of claims 66-85, wherein said suppression of an immune response is brought about by or manifests itself in inhibition of the interaction between said cell and other cells, wherein said interaction would normally lead to an immune response.

90. The composition of any one of claims 66-85, wherein said suppression of the immune response is brought about by or manifests itself in the killing of said cells.

91. The composition of claim 90, wherein said killing is mediated by binding of a plurality of antigen-binding domains, wherein said antibody-based antigen-binding domains are part of a multivalent polypeptide, and where neither cytotoxic entities nor immunological mechanisms are needed to causes or leads to said killing.

92. The composition of any one of claims 66-91, formulated in a pharmaceutically acceptable carrier and/or diluent

93. A pharmaceutical preparation comprising the composition of claim 75 in an amount sufficient to suppress an immune response in an animal.

94. A pharmaceutical preparation comprising the composition of claim 76 in an amount sufficient to inhibit IL-2 secretion in an animal.

95. A pharmaceutical preparation comprising the composition of claim 77 in an amount sufficient to inhibit T cell proliferation in an animal.

96. The use of a composition of any one of claims 66-91, for preparing a pharmaceutical preparation for the treatment of animals, such as where said animals are human.

97. A nucleic acid including a protein coding sequence for a polypeptide of the composition of any of claims 66-91.

98. A vector comprising the coding sequence of claim 97, and a transcriptional regulatory sequence operably linked thereto.

99. A host cell harboring a nucleic acid of claim 97 or the vector of claim 98.

100. A method for the production of an immunosuppressive composition, comprising culturing the cells of claim 99 under conditions wherein the nucleic acid is expressed.

101. A method for suppressing activation of a cell of the immune system, such as expressing HLA DR, comprising treating the cell with a composition of any of claims 66-92.

102. A method for suppressing proliferation of a cell of the immune system, such as expressing HLA DR, comprising treating the cell with a composition of any of claims 66-92.

103. A method for suppressing IL-2 secretion by a cell of the immune system, such as expressing HLA DR, comprising treating the cell with a composition of any of claims 66-92

104. A method for immunosuppressing a patient, comprising administering to the patient an effective amount of a composition of any of claims 66-92 to reduce the level of immunological responsiveness in the patient.

105. A method for killing a cell expressing an antigen on the surface of said cell comprising the step of treating the cell with a plurality of antigen-binding domains of any one of claims 66-87, wherein said antibody-based antigen-binding domains are part of a multivalent polypeptide, and where neither cytotoxic entities nor immunological mechanisms are needed to causes or leads to said killing, such where said antigen is HLA DR.

106. The use according to claim 96, wherein said treatment is the treatment of a disorder selected from rheumatoid arthritis, juvenile arthritis, multiple sclerosis, Grave's disease, insulin-dependent diabetes, narcolepsy, psoriasis, systemic lupus erythematosus, ankylosing spondylitis, transplant rejection, graft vs. host disease, Hashimoto's disease, myasthenia gravis, pemphigus vulgaris, glomerulonephritis, thyroiditis, pancreatitis, insulitis, primary biliary cirrhosis, irritable bowel disease and Sjogren syndrome.

107. The use according to claim 96, wherein said treatment is the treatment of a disorder selected from myasthenia gravis, rheumatoid arthritis, multiple sclerosis, transplant rejection and graft vs. host disease.

108 A method of suppressing the interaction of a cell of the immune system with an other cell, comprising contacting the cell with the composition of any of claims 66-92.

109. A method for conducting a pharmaceutical business comprising:

(i) isolating one or more antigen-binding domains that bind to antigens expressed on the surface of human cells;

(ii) generating a multivalent composition, such as multivalent polypeptide, comprising a plurality of said antigen-binding domains, which multivalent composition kills with an EC_{50} of 50nM or less transformed or activated cells that express said antigen, where neither cytotoxic entities nor immunological mechanisms are needed to cause or lead to said killing.;

(iii) conducting therapeutic profiling of the multivalent composition, for efficacy and toxicity in animals;

(iv) preparing a package insert describing the multivalent composition for treatment of proliferative disorders; and

(v) marketing the multivalent composition for treatment of proliferative disorders.

110. A method for conducting a life science business comprising:

(i) isolating one or more antigen-binding domains that bind to antigens expressed on the surface of human cells;

(ii) generating a multivalent composition, such as multivalent polypeptide, comprising a plurality of said antigen-binding domains, which multivalent composition kills with an EC_{50} of 50nM or less transformed or activated cells expressing said antigen where neither cytotoxic entities nor immunological mechanisms are needed to cause or lead to said killing;

(iii) licensing, jointly developing or selling, to a third party, the rights for selling the multivalent composition.

111. The method of any of claims 109 or 110, wherein the antigen-binding domain is isolated by a method which includes

a. isolation of VL and VH domains of human composition from a recombinant antibody library by ability to bind to HLA DR,

b. generating a library of variants at least one of the CDR1, CDR2 and CDR3 sequences of one or both of the VL and VH domains, and

c. isolation of VL and VH domains from the library of variants by ability bind to HLA DR with a K_d of 1μM or less.

112. A method for conducting a pharmaceutical business comprising:

(i) isolating one or more antigen-binding domains that bind to MHC class II expressed on the surface of human cells with a K_d of 1μM or less;

(ii) generating a composition comprising said antigen-binding domains, which composition is immunosuppressant with an IC_{50} of 100nM or less;

(iii) conducting therapeutic profiling of the composition for efficacy and toxicity in animals;

(iv) preparing a package insert describing the use of the composition for immunosuppression therapy; and

(v) marketing the composition for use as an immunosuppressant.

113. A method for conducting a life science business comprising:

(i) isolating one or more antigen-binding domains that bind to MHC class II expressed on the surface of human cells with a K_d of 1μM or less;

(ii) generating a composition comprising said antigen-binding domains, which composition is immunosuppressant with an IC_{50} of 100nM or less;

(iii) licensing, jointly developing or selling, to a third party, the rights for selling the composition.

114. The method of any of claims 112 or 113, wherein the antigen-binding domain is isolated by a method which includes

a. isolation of VL and VH domains of human composition from a recombinant antibody library by ability to bind to HLA DR,

b. generating a library of variants at least one of the CDR1, CDR2 and CDR3 sequences of one or both of the VL and VH domains, and

c. isolation of VL and VH domains from the library of variants by ability to bind to HLA DR with a Kd of 1μM or less.

115. The method of any of claims 109-114, wherein said antigen-binding domain comprises a combination of VH and VL domains found in the clones taken from the list of MS-GPC-1, MS-GPC-8, MS-GPC-10, MS-GPC-8-1, MS-GPC-8-6, MS-GPC-8-9, MS-GPC-8-10, MS-GPC-8-17, MS-GPC-8-18, MS-GPC-8-27, MS-GPC-8-6-2, MS-GPC-8-6-19, MS-GPC-8-6-27, MS-GPC-8-6-45, MS-GPC-8-6-13, MS-GPC-8-6-47, MS-GPC-8-10-57, MS-GPC-8-27-7, MS-GPC-8-27-10 and MS-GPC-8-27-41.

Figure 1a

	MS-GPC-8-27-7	MS-GPC-8-27-10	MS-GPC-8-6-13	MS-GPC-8-27-41	MS-GPC-8-6-47	MS-GPC-8-10-57	MS-GPC-8-6-27	MS-GPC-8	MS-GPC-8-6
Plastic	-0,004	-0,02	-0,022	-0,025	-0,001	0,005	0,007	-0,022	-0,018
BSA	-0,003	-0,019	-0,021	-0,022	0,008	0,003	0,003	-0,016	-0,019
Testosterone -BSA	-0,005	-0,01	-0,012	-0,007	0,011	0,003	0,002	-0,009	-0,012
Lysozyme	-0,005	-0,079	-0,079	-0,073	0,013	0,014	0,006	-0,081	-0,072
human Apotransferrin	-0,009	-0,016	-0,018	-0,018	-0,005	-0,008	-0,004	-0,014	-0,016
MHCII (DRA*0101/ DRB1*0401)	1,549	1,493	1,467	1,525	1,4	1,256	1,297	1,058	1,306

Figure 1b

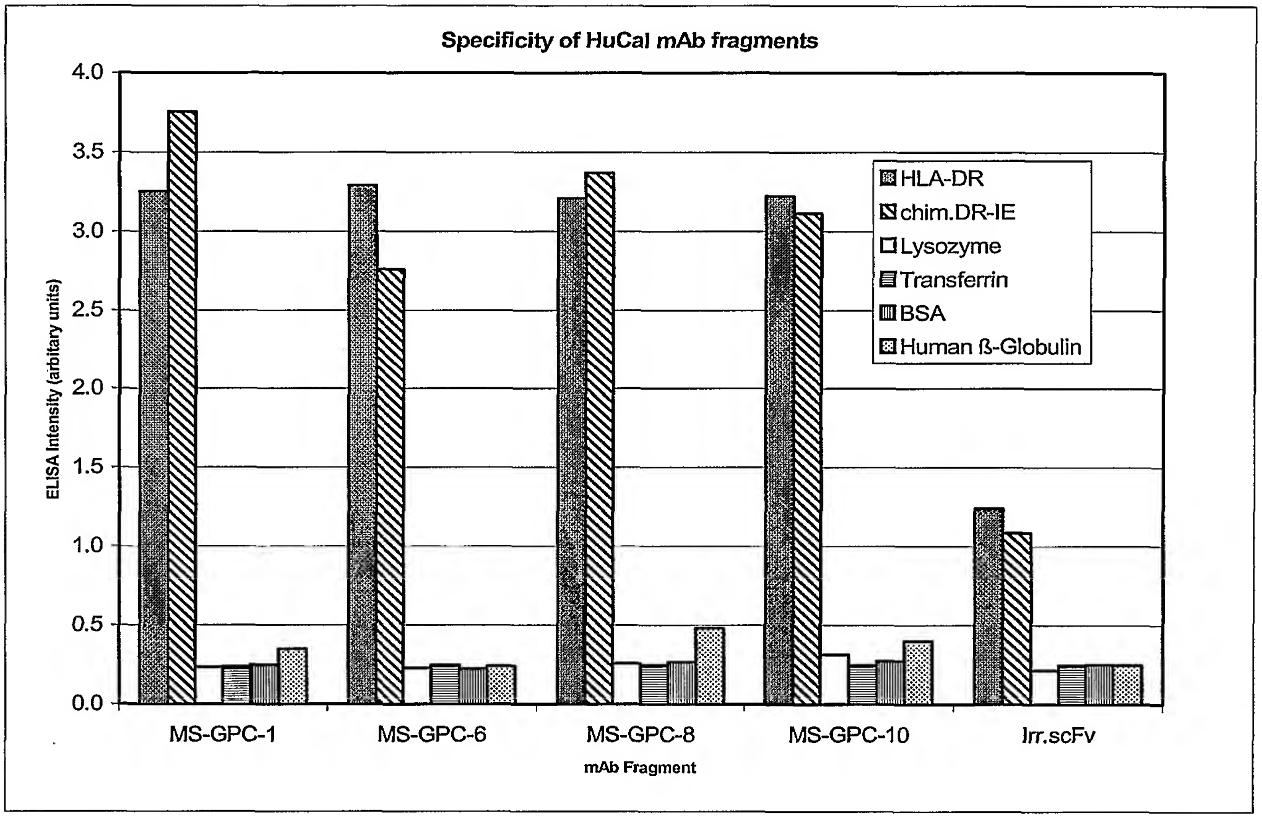

Specificity of HuCal mAb fragments
ELISA Intensity (arbitary units)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HLA-DR
chim.DR-IE
Lysozyme
Transferrin
BSA
Human ß-Globulin
MS-GPC-1
MS-GPC-6
MS-GPC-8
MS-GPC-10
Irr.scFv
mAb Fragment

Figure 2

Cell line	HLA-	DRB1*	scFv				IgG			
			MS-GPC-1	MS-GPC-6	MS-GPC-8	MS-GPC-10	MS-GPC-8	MS-GPC-8-10-57	MS-GPC-8-27-41	MS-GPC-8-6-13
LG2	DR1	0101	+	+	+	+	+	+	+	+
E4181324	DR2	15021	+	+	+	+	+	+	+	+
VAVY	DR3	0301	+	+	+	+	+	+	+	+
Priess	DR4Dw4	0401	+	+	+	+	+	+	+	+
TS10	DR4Dw10	0402	+	+	+	+	+	+	+	+
BIN40	DR4Dw14	0404	+	+	+	+	+	+	+	+
WT47	DR6	1302	+	+	+	+	+	+	+	+
TEM	DR6	1401	+	+	+	+	+	+	+	+
TAB089	DR8	8031	+	+	+	+	+	+	+	+
DKB	DR9	9012	+	+	+	+	+	+	+	+
L257.6	DRw53	B4*0101	+/-	+	+	+/-	nt	nt	nt	nt
L105.1	DRw52	B3*0101	+	+	+	+	nt	nt	nt	nt
L25.4	DPw4/w4.2	DP0103/0402	-	+	-	-	nt	nt	nt	nt
L256.12	DPw2/w2.1	DP0202/0201	-	+/-	-	-	nt	nt	nt	nt
L21.3	DQ7/w2	DQ0201/0602	-	+	+	-	nt	nt	nt	nt

Figure 3

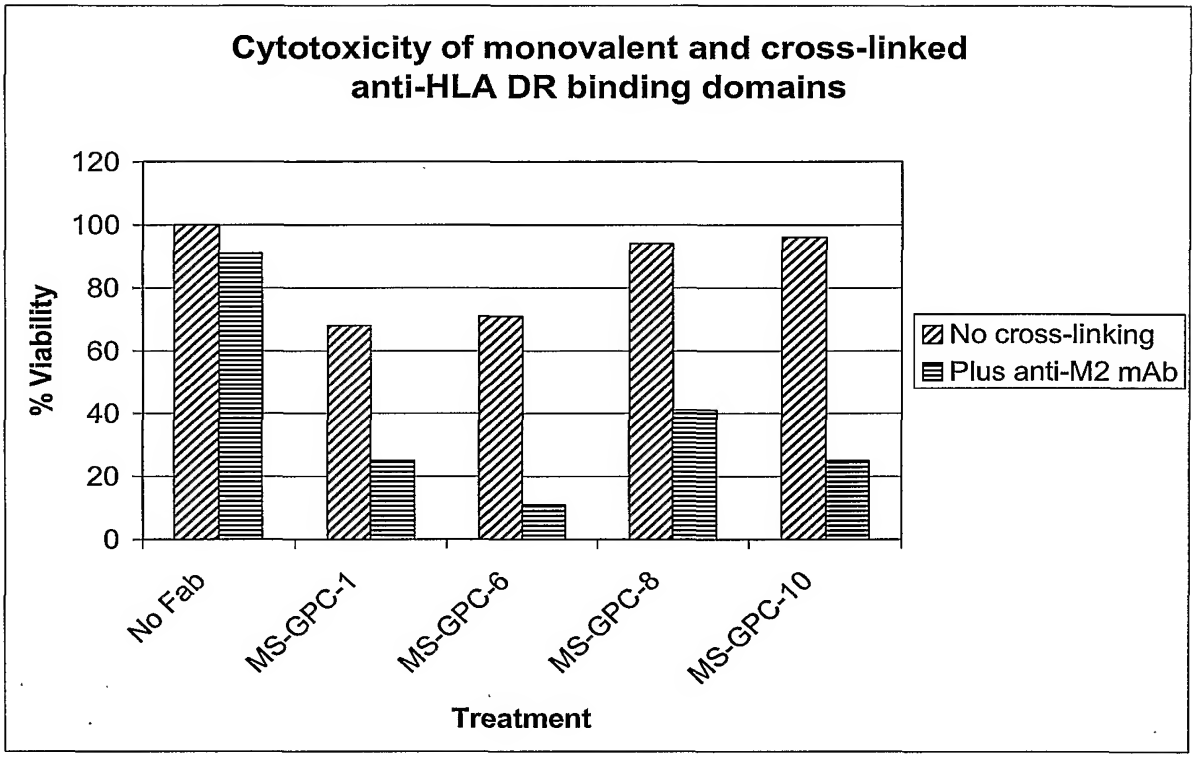

Cytotoxicity of monovalent and cross-linked anti-HLA DR binding domains
120
100
80
60
40
20
0
% Viability
No cross-linking
Plus anti-M2 mAb
No Fab
MS-GPC-1
MS-GPC-6
MS-GPC-8
MS-GPC-10
Treatment

Figure 4

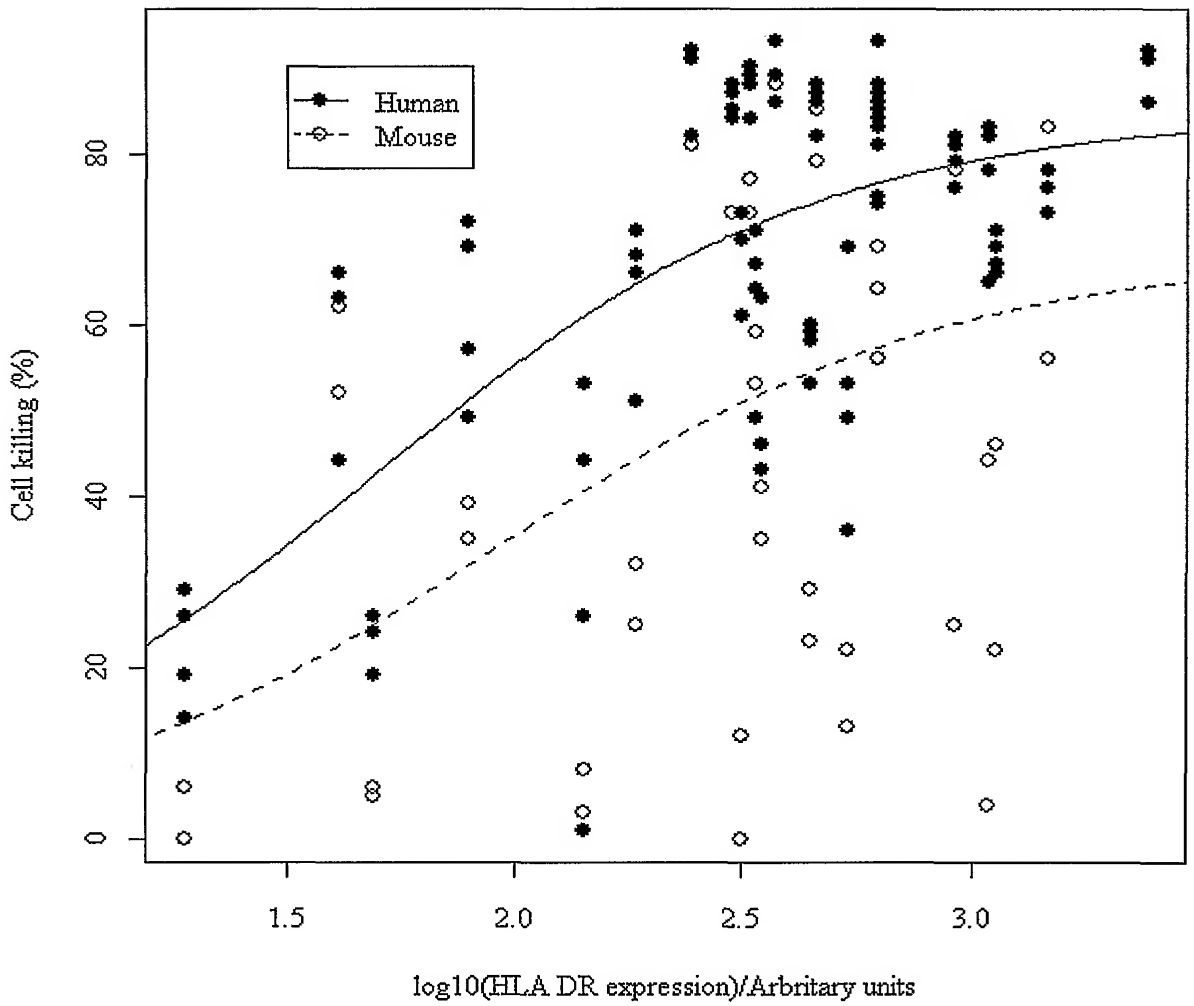

Human
Mouse
Cell killing (%)
0
20
40
60
80
1.5
2.0
2.5
3.0
log10(HLA DR expression)/Arbitrary units

Figure 5

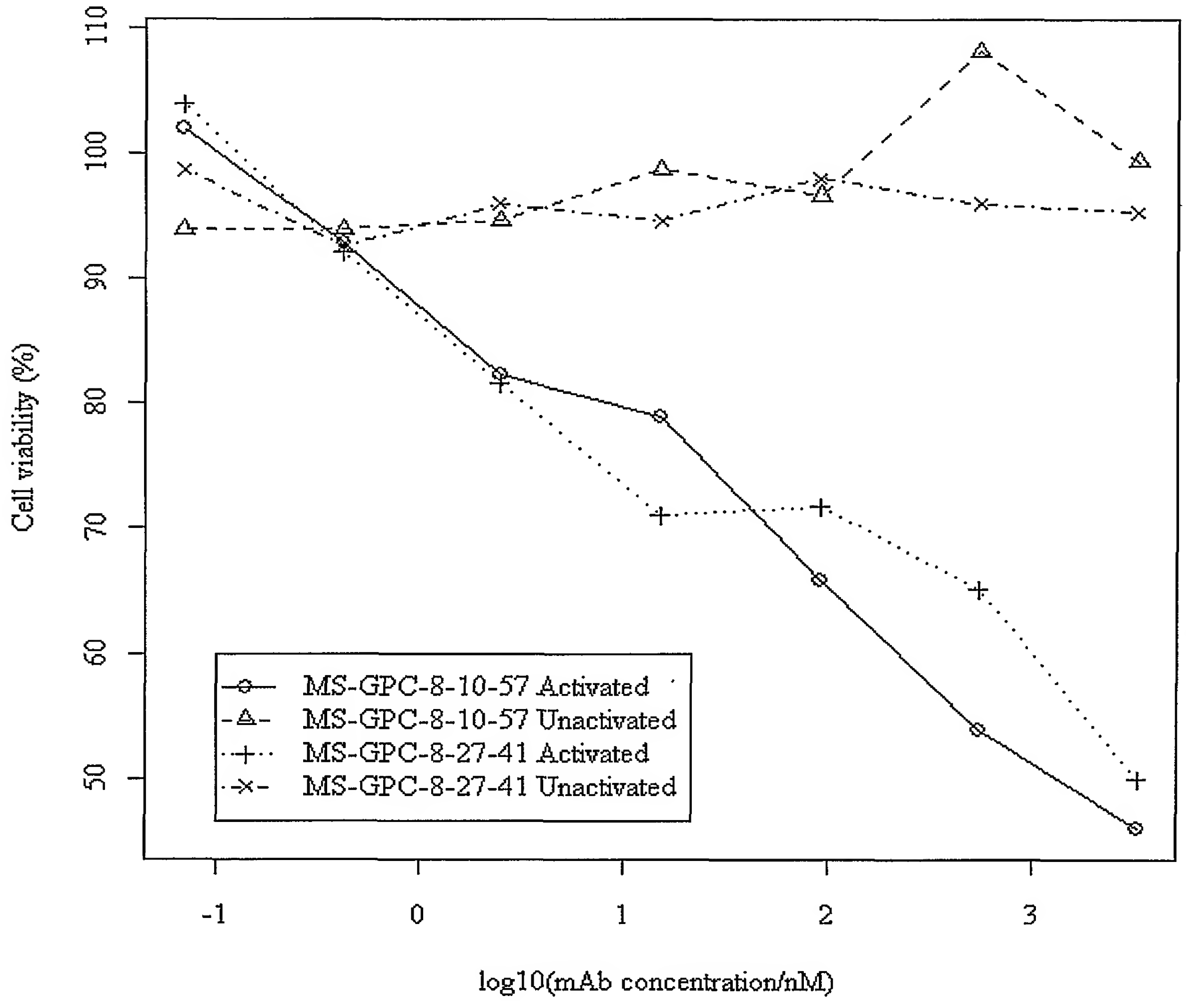

110
100
90
80
70
60
50
Cell viability (%)
-1
0
1
2
3
log10(mAb concentration/nM)
MS-GPC-8-10-57 Activated
MS-GPC-8-10-57 Unactivated
MS-GPC-8-27-41 Activated
MS-GPC-8-27-41 Unactivated

Figure 6

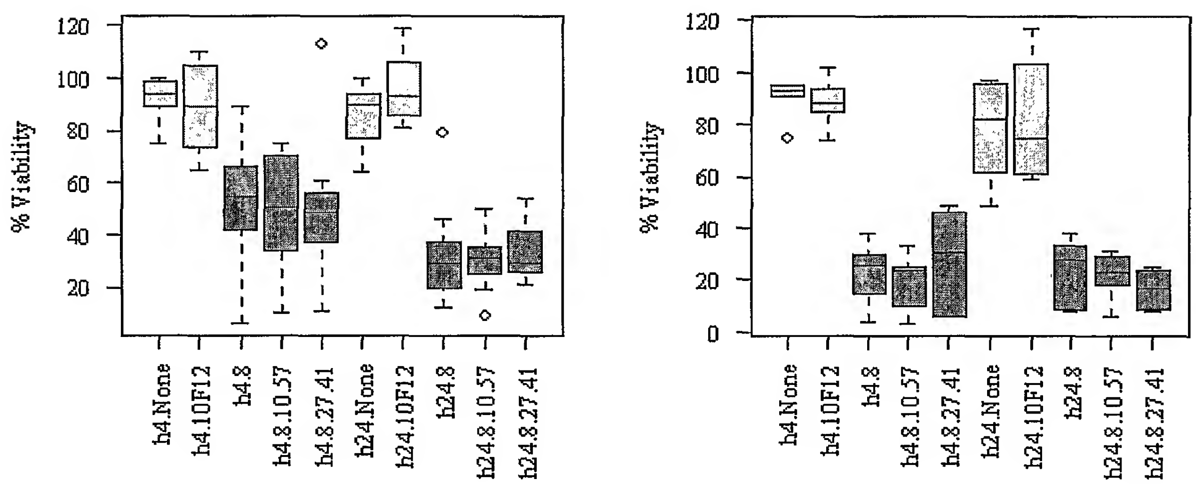

% Viability
120
100
80
60
40
20
0
h4.None
h4.10F12
h4.8
h4.8.10.57
h4.8.27.41
h24.None
h24.10F12
h24.8
h24.8.10.57
h24.8.27.41

Figure 7a

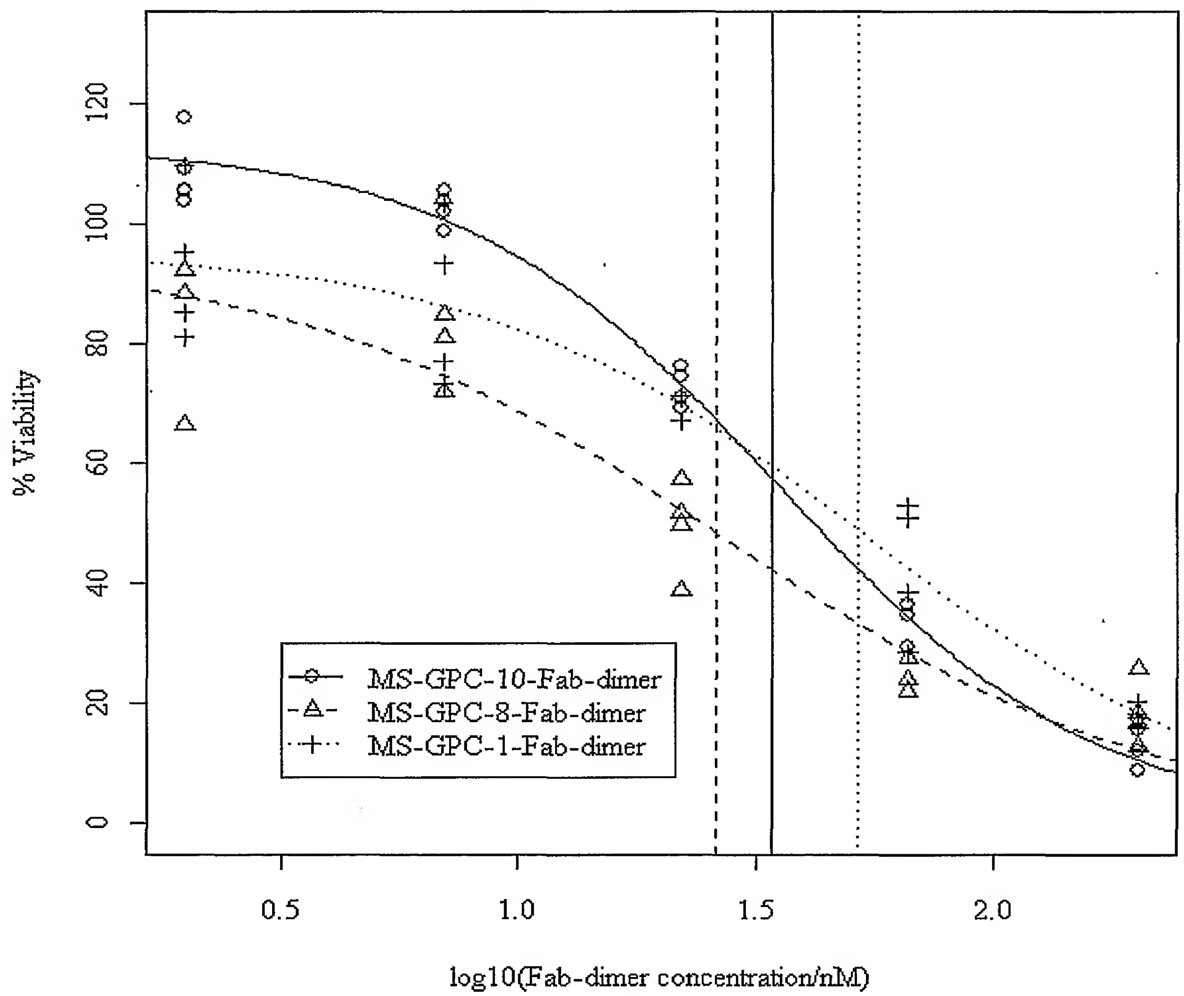

120
100
80
60
40
20
0
% Viability
0.5
1.0
1.5
2.0
log10(Fab-dimer concentration/nM)
MS-GPC-10-Fab-dimer
MS-GPC-8-Fab-dimer
MS-GPC-1-Fab-dimer

Figure 7b

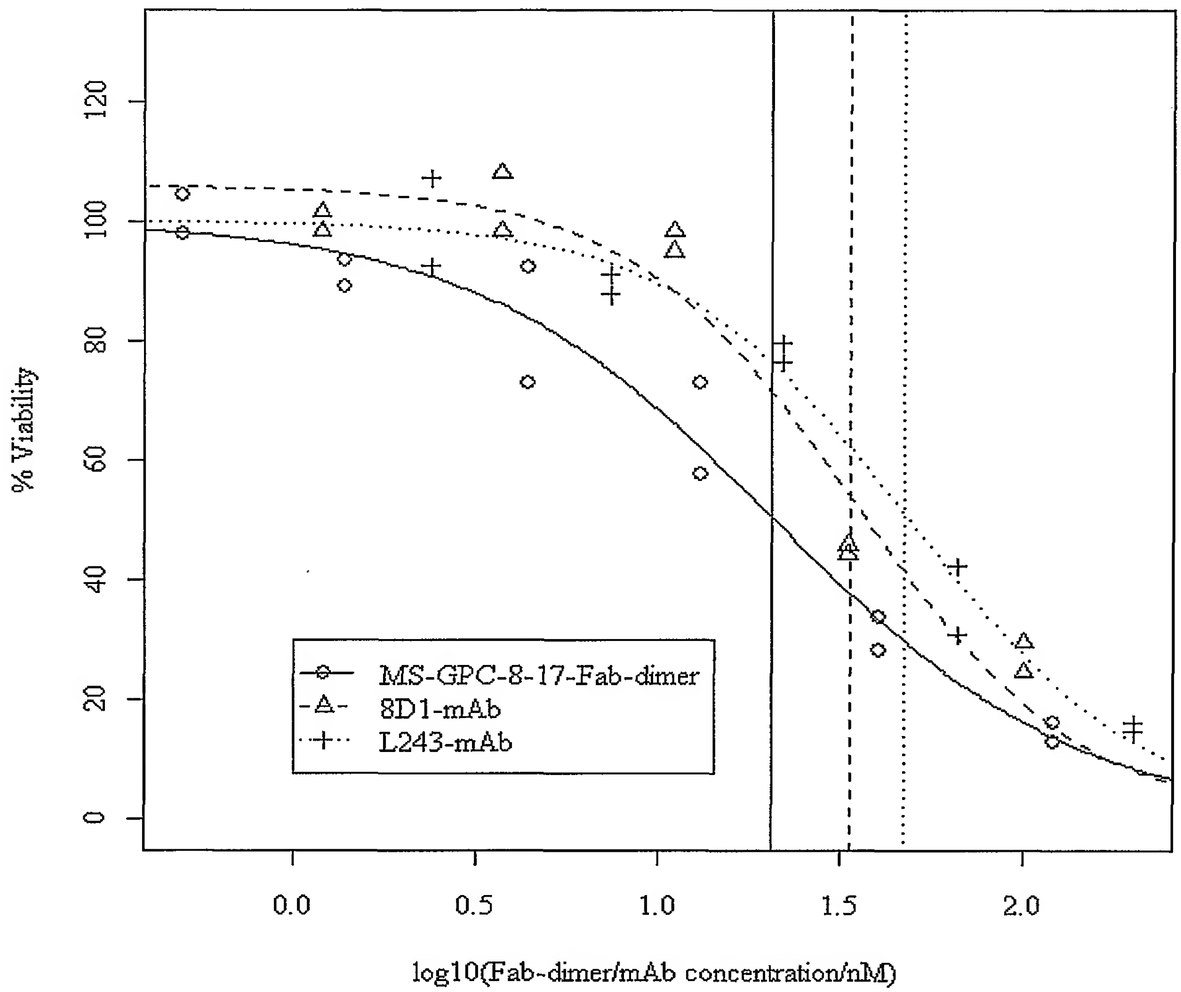

% Viability
0
20
40
60
80
100
120
0.0
0.5
1.0
1.5
2.0
log10(Fab-dimer/mAb concentration/nM)
MS-GPC-8-17-Fab-dimer
8D1-mAb
L243-mAb

Figure 7c

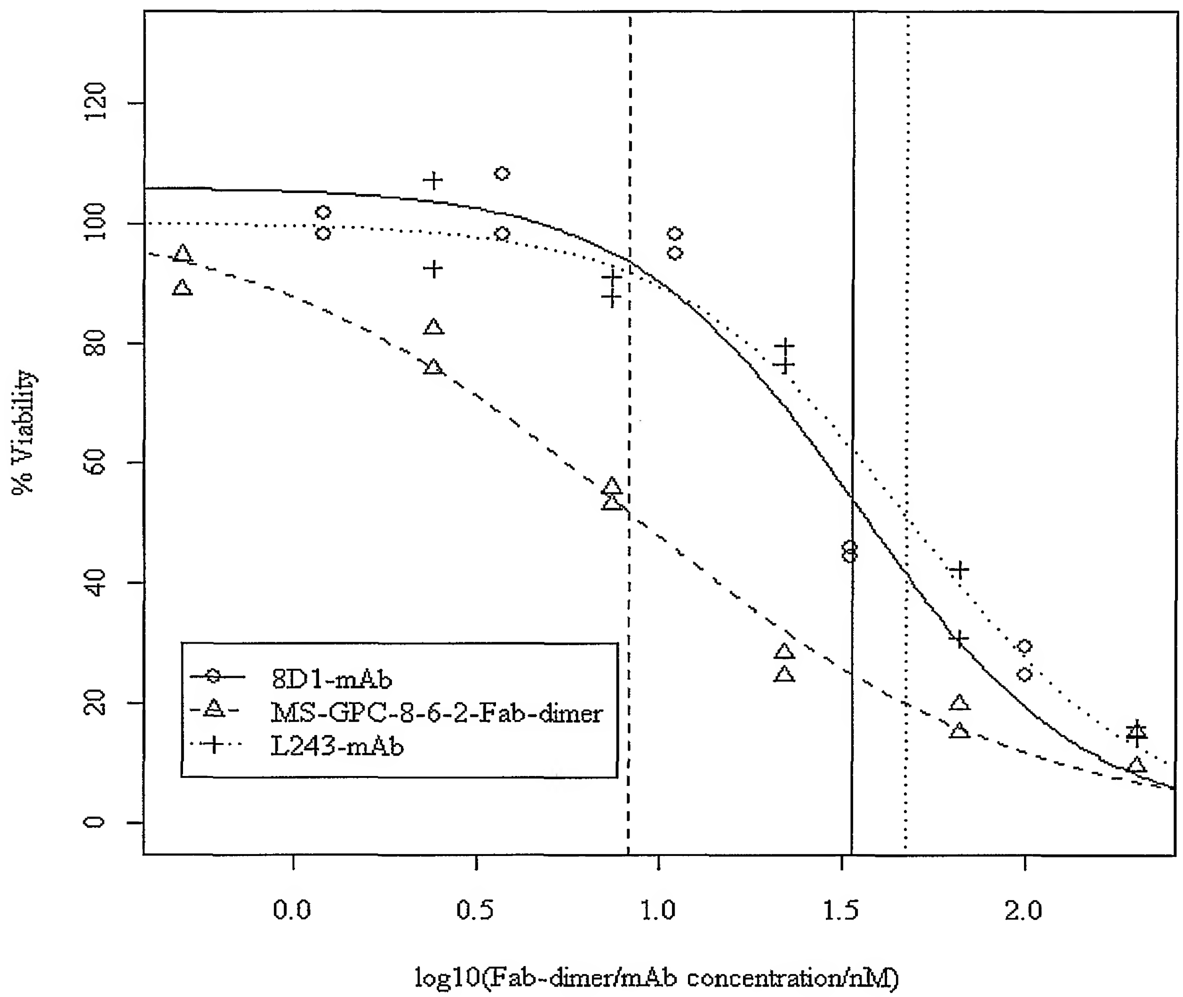
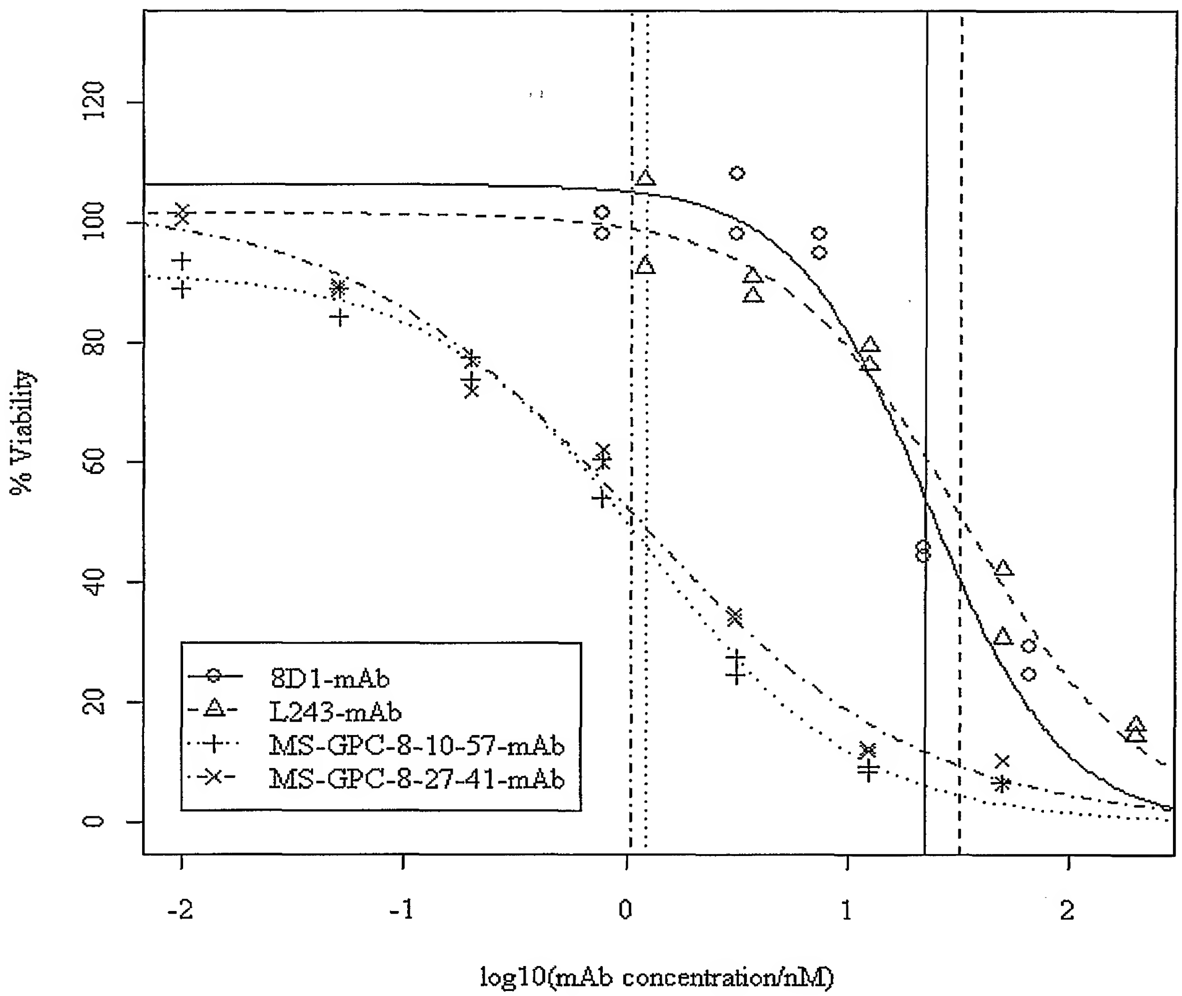

% Viability
0
20
40
60
80
100
120
0.0
0.5
1.0
1.5
2.0
log10(Fab-dimer/mAb concentration/nM)
8D1-mAb
MS-GPC-8-6-2-Fab-dimer
L243-mAb

Figure 7d



120
100
80
60
40
20
0
% Viability
8D1-mAb
L243-mAb
MS-GPC-8-10-57-mAb
MS-GPC-8-27-41-mAb
-2
-1
0
1
2
log10(mAb concentration/nM)

Figure 8a

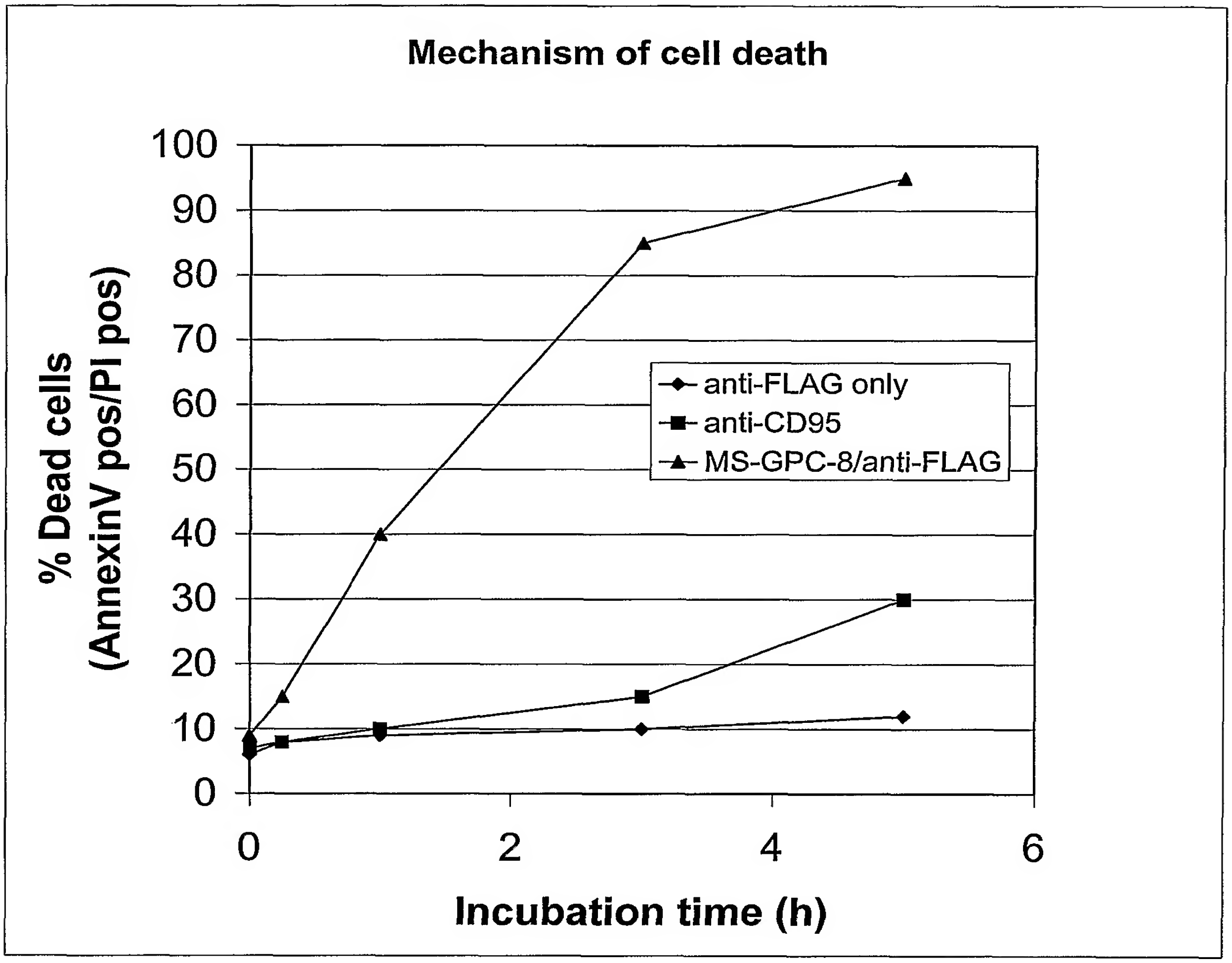

Mechanism of cell death
% Dead cells
(AnnexinV pos/PI pos)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
Incubation time (h)
anti-FLAG only
anti-CD95
MS-GPC-8/anti-FLAG

Figure 8b

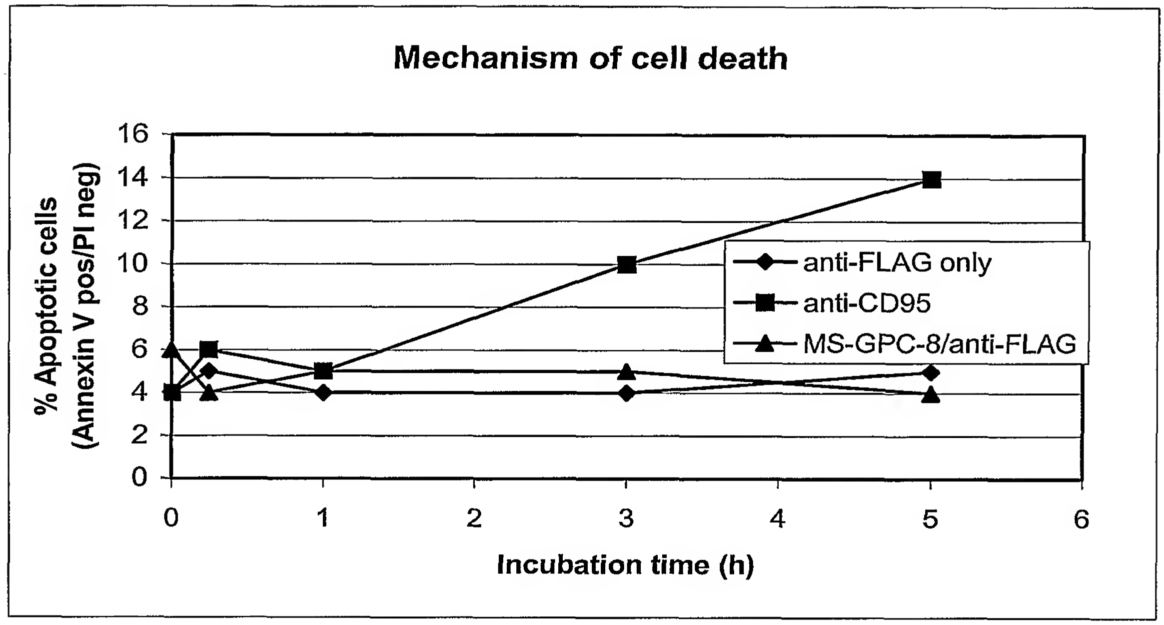

Mechanism of cell death
% Apoptotic cells
(Annexin V pos/PI neg)
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
Incubation time (h)
anti-FLAG only
anti-CD95
MS-GPC-8/anti-FLAG

Figure 9a

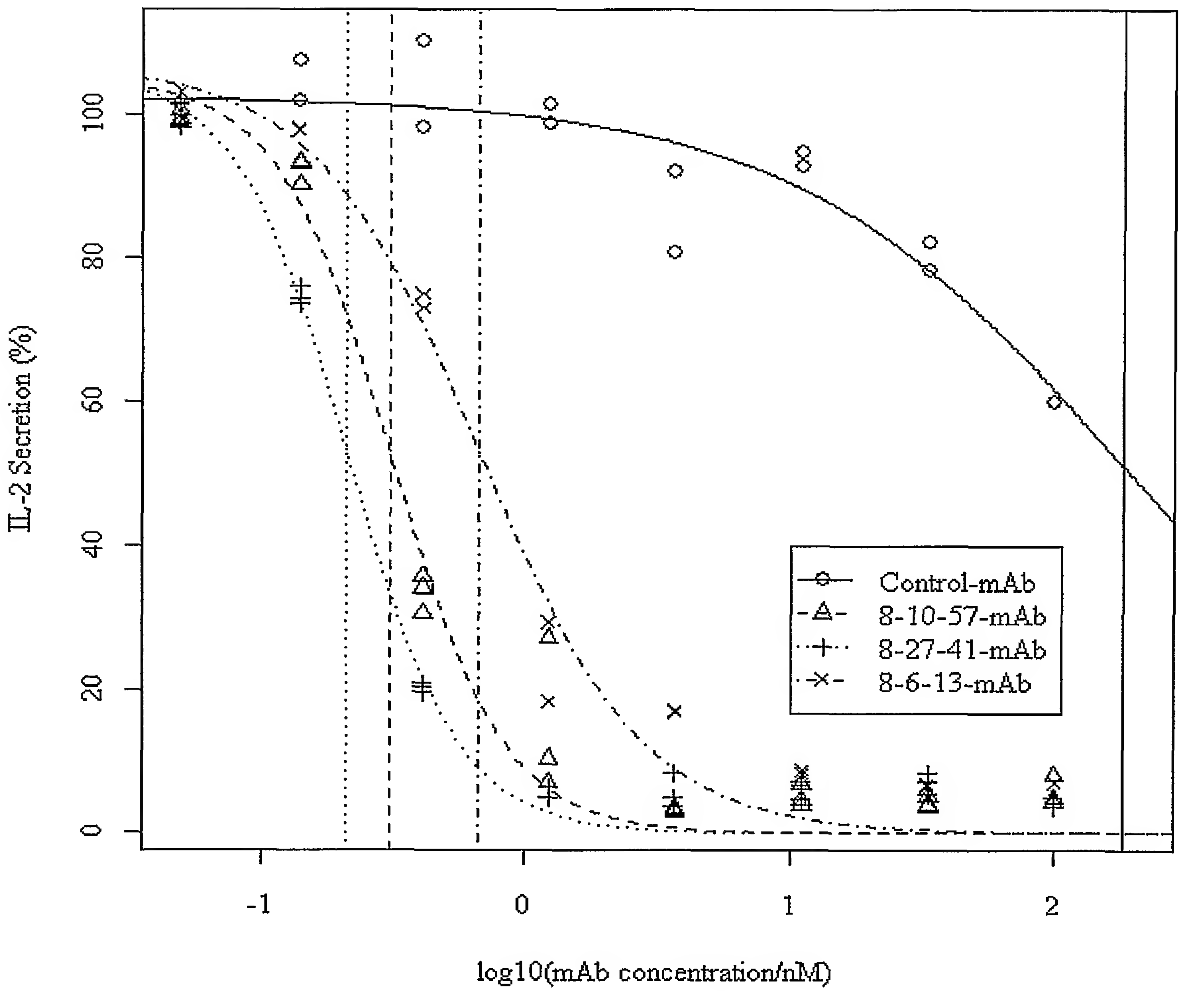

IL-2 Secretion (%)
0
20
40
60
80
100
-1
0
1
2
log10(mAb concentration/nM)
Control-mAb
8-10-57-mAb
8-27-41-mAb
8-6-13-mAb

Figure 9b

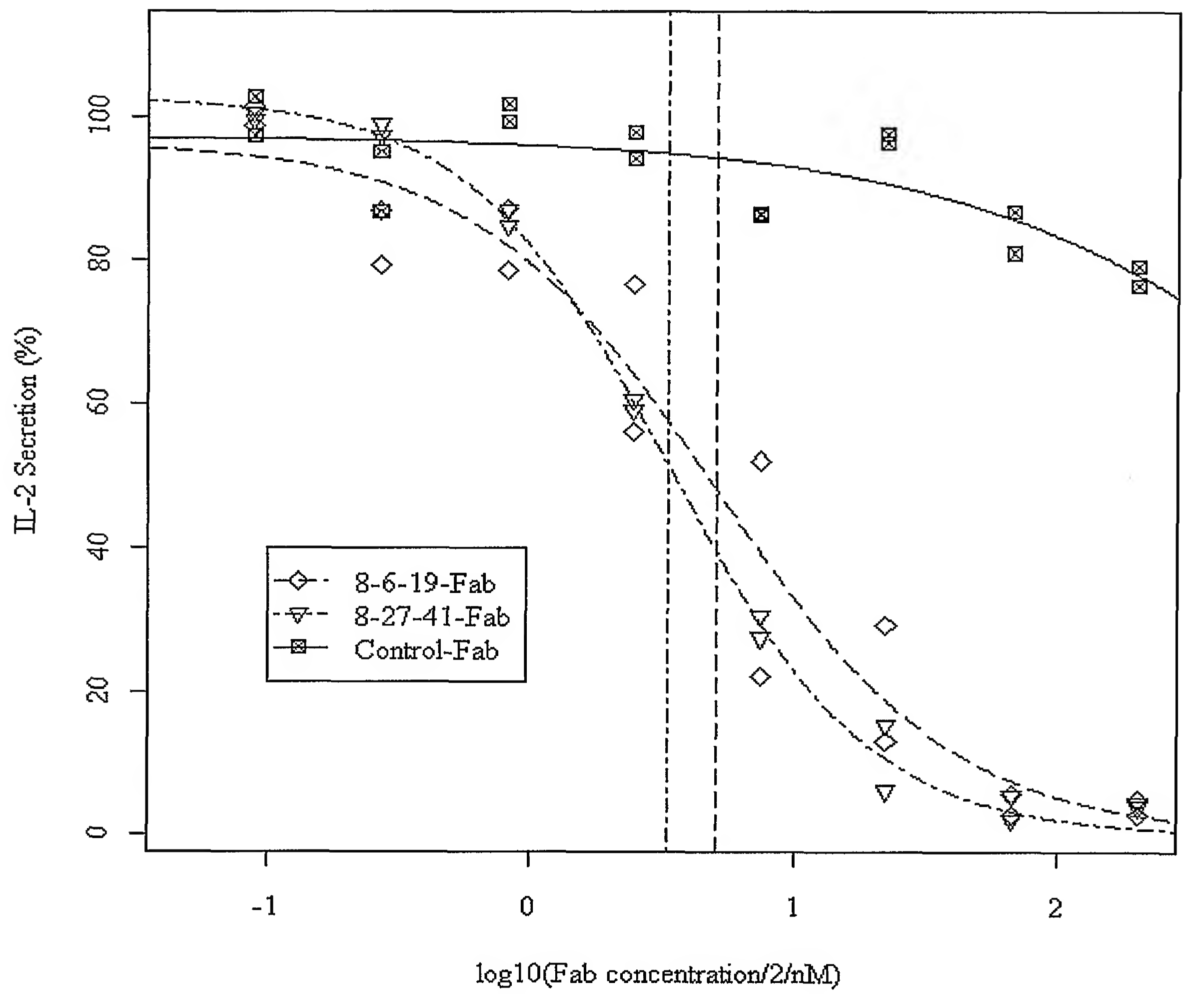
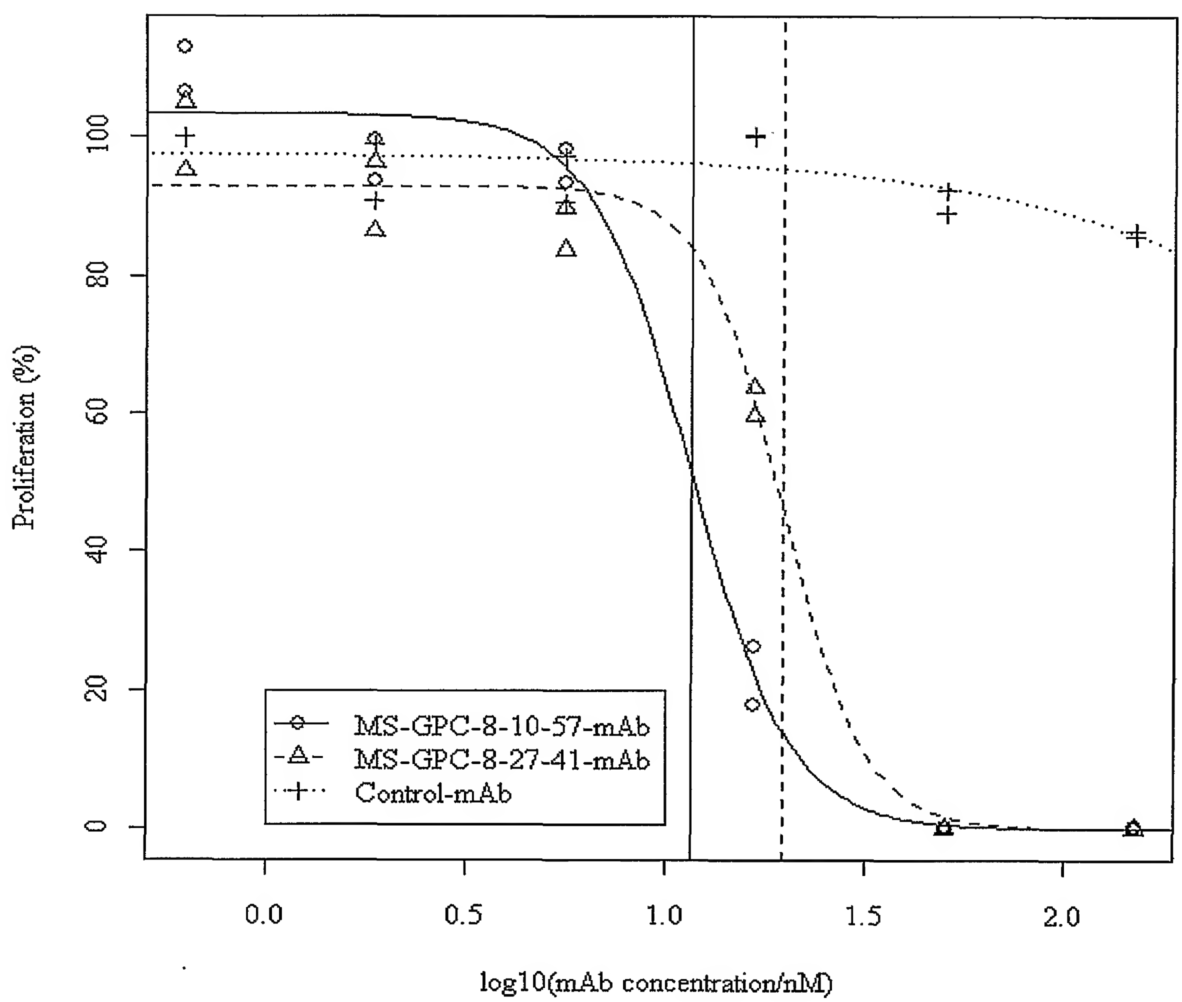

100
80
60
40
20
0
IL-2 Secretion (%)
8-6-19-Fab
8-27-41-Fab
Control-Fab
-1
0
1
2
log10(Fab concentration/2/nM)

Figure 10



Proliferation (%)
0
20
40
60
80
100
0.0
0.5
1.0
1.5
2.0
log10(mAb concentration/nM)
MS-GPC-8-10-57-mAb
MS-GPC-8-27-41-mAb
Control-mAb

Figure 11

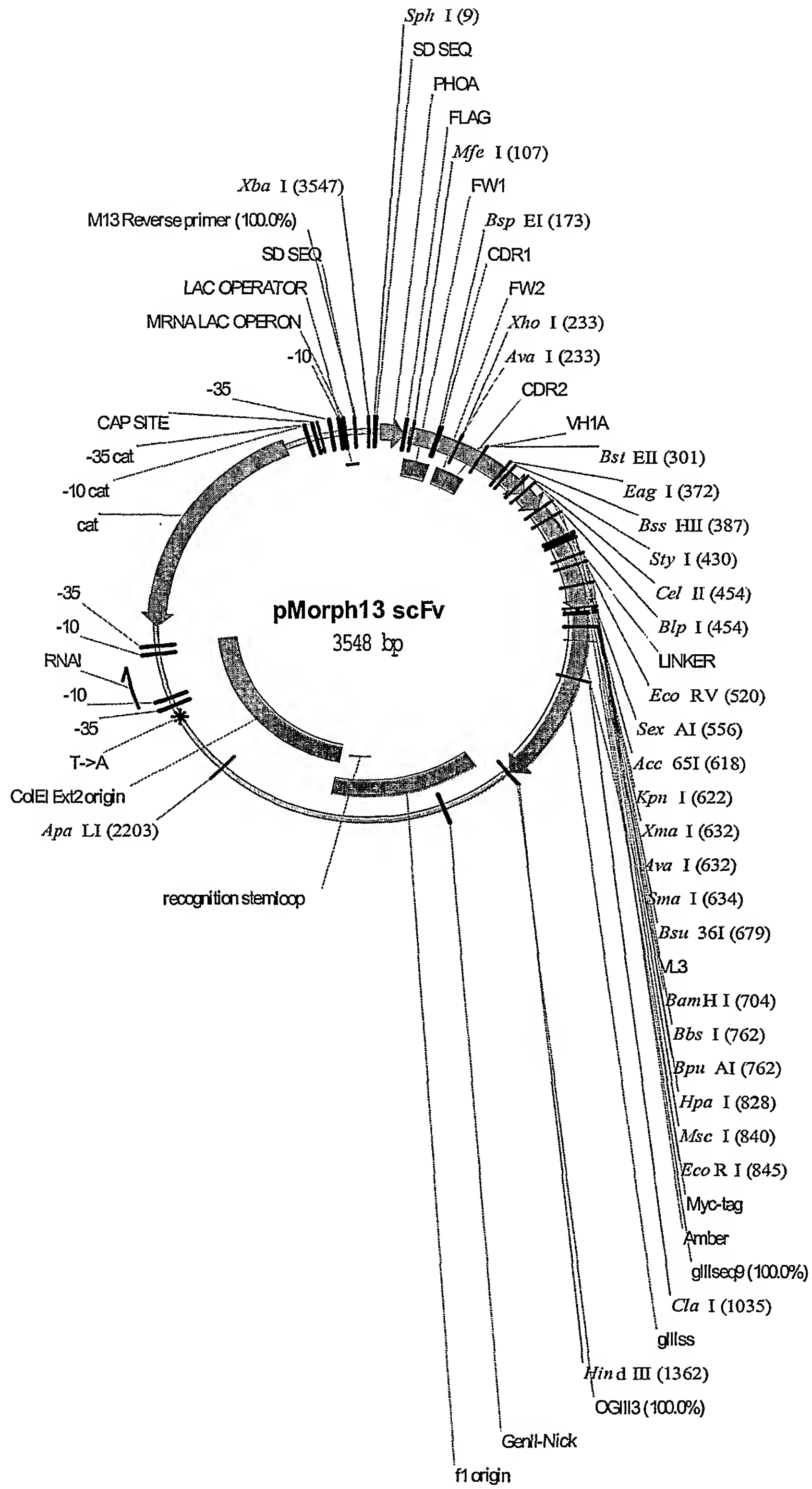

Sph I (9)
SD SEQ
PHOA
FLAG
Mfe I (107)
FW1
Bsp EI (173)
CDR1
FW2
Xho I (233)
Ava I (233)
CDR2
VH1A
Bst EII (301)
Eag I (372)
Bss HII (387)
Sty I (430)
Cel II (454)
Blp I (454)
LINKER
Eco RV (520)
Sex AI (556)
Acc 65I (618)
Kpn I (622)
Xma I (632)
Ava I (632)
Sma I (634)
Bsu 36I (679)
VL3
BamH I (704)
Bbs I (762)
Bpu AI (762)
Hpa I (828)
Msc I (840)
EcoR I (845)
Myc-tag
Amber
gIIIseq9 (100.0%)
Cla I (1035)
gIIIss
Hind III (1362)
OGIII3 (100.0%)
GenII-Nick
f1 origin
recognition stemloop
Apa LI (2203)
ColEI Ext2 origin
T->A
-35
-10
RNAI
-10
-35
cat
-10 cat
-35 cat
CAP SITE
-35
-10
MRNA LAC OPERON
LAC OPERATOR
SD SEQ
M13 Reverse primer (100.0%)
Xba I (3547)
pMorph13 scFv
3548 bp

Figure 11 (cont)

```
    XbaISphI
    ~~~~~~~~~~
  1 AGAGCATGCG TAGGAGAAAA TAAAATGAAA CAAAGCACTA TTGCACTGGC
    TCTCGTACGC ATCCTCTTTT ATTTTACTTT GTTTCGTGAT AACGTGACCG

 51 ACTCTTACCG TTGCTCTTCA CCCCTGTTAC CAAAGCCGAC TACAAAGATG
    TGAGAATGGC AACGAGAAGT GGGGACAATG GTTTCGGCTG ATGTTTCTAC

           MfeI
          ~~~~~~~
101 AAGTGCAATT GGTTCAGTCT GGCGCGGAAG TGAAAAAACC GGGCAGCAGC
    TTCACGTTAA CCAAGTCAGA CCGCGCCTTC ACTTTTTTGG CCCGTCGTCG

                             BspEI
                            ~~~~~~
151 GTGAAAGTGA GCTGCAAAGC CTCCGGAGGC ACTTTTAGCA GCTATGCGAT
    CACTTTCACT CGACGTTTCG GAGGCCTCCG TGAAAATCGT CGATACGCTA

                                        XhoI
                                       ~~~~~~
                                        AvaI
                                       ~~~~~~
201 TAGCTGGGTG CGCCAAGCCC CTGGGCAGGG TCTCGAGTGG ATGGGCGGCA
    ATCGACCCAC GCGGTTCGGG GACCCGTCCC AGAGCTCACC TACCCGCCGT

                                                   BstEII
                                                        ~
251 TTATTCCGAT TTTTGGCACG GCGAACTACG CGCAGAAGTT TCAGGGCCGG
    AATAAGGCTA AAAACCGTGC CGCTTGATGC GCGTCTTCAA AGTCCCGGCC

    BstEII
    ~~~~~~
301 GTGACCATTA CCGCGGATGA AAGCACCAGC ACCGCGTATA TGGAACTGAG
    CACTGGTAAT GGCGCCTACT TTCGTGGTCG TGGCGCATAT ACCTTGACTC

                            EagI           BssHII
                           ~~~~~~         ~~~~~~~
351 CAGCCTGCGT AGCGAAGATA CGGCCGTGTA TTATTGCGCG CGTTATTATG
    GTCGGACGCA TCGCTTCTAT GCCGGCACAT AATAACGCGC GCAATAATAC

                                   StyI
                                  ~~~~~~~
401 ATCGTATGTA TAATATGGAT TATTGGGGCC AAGGCACCCT GGTGACGGTT
    TAGCATACAT ATTATACCTA ATAACCCCGG TTCCGTGGGA CCACTGCCAA

      BlpI
     ~~~~~~~
      CelII
     ~~~~~~~
451 AGCTCAGCGG GTGGCGGTTC TGGCGGCGGT GGGAGCGGTG GCGGTGGTTC
    TCGAGTCGCC CACCGCCAAG ACCGCCGCCA CCCTCGCCAC CGCCACCAAG

                      EcoRV
                     ~~~~~~~
501 TGGCGGTGGT GGTTCCGATA TCGAACTGAC CCAGCCGCCT TCAGTGAGCG
```

```
     ACCGCCACCA CCAAGGCTAT AGCTTGACTG GGTCGGCGGA AGTCACTCGC

            SexAI
          ~~~~~~~~~
551  TTGCACCAGG TCAGACCGCG CGTATCTCGT GTAGCGGCGA TGCGCTGGGC
     AACGTGGTCC AGTCTGGCGC GCATAGAGCA CATCGCCGCT ACGCGACCCG

                                          XmaI
                                         ~~~~~~
                           KpnI           SmaI
                         ~~~~~~~~        ~~~~~~
                          Acc65I          AvaI
                         ~~~~~~~~        ~~~~~~
601  GATAAATACG CGAGCTGGTA CCAGCAGAAA CCCGGGCAGG CGCCAGTTCT
     CTATTTATGC GCTCGACCAT GGTCGTCTTT GGGCCCGTCC GCGGTCAAGA

                                     Bsu36I
                                   ~~~~~~~~~
651  GGTGATTTAT GATGATTCTG ACCGTCCCTC AGGCATCCCG GAACGCTTTA
     CCACTAAATA CTACTAAGAC TGGCAGGGAG TCCGTAGGGC CTTGCGAAAT

        BamHI
       ~~~~~~
701  GCGGATCCAA CAGCGGCAAC ACCGCGACCC TGACCATTAG CGGCACTCAG
     CGCCTAGGTT GTCGCCGTTG TGGCGCTGGG ACTGGTAATC GCCGTGAGTC

         BpuAI
        ~~~~~~
         BbsI
        ~~~~~~
751  GCGGAAGACG AAGCGGATTA TTATTGCCAG AGCTATGACG CTCATATGCG
     CGCCTTCTGC TTCGCCTAAT AATAACGGTC TCGATACTGC GAGTATACGC

                                  HpaI          MscI     EcoRI
                                 ~~~~~~       ~~~~~~~~  ~~~~~~
801  TCCTGTGTTT GGCGGCGGCA CGAAGTTAAC CGTTCTTGGC CAGGAATTCG
     AGGACACAAA CCGCCGCCGT GCTTCAATTG GCAAGAACCG GTCCTTAAGC

851  AGCAGAAGCT GATCTCTGAG GAGGATCTGA ACTAGGGTGG TGGCTCTGGT
     TCGTCTTCGA CTAGAGACTC CTCCTAGACT TGATCCCACC ACCGAGACCA

901  TCCGGTGATT TTGATTATGA AAAGATGGCA AACGCTAATA AGGGGGCTAT
     AGGCCACTAA AACTAATACT TTTCTACCGT TTGCGATTAT TCCCCCGATA
                                  gIIIseq9  100.0%
                               ======================

951  GACCGAAAAT GCCGATGAAA ACGCGCTACA GTCTGACGCT AAAGGCAAAC
     CTGGCTTTTA CGGCTACTTT TGCGCGATGT CAGACTGCGA TTTCCGTTTG

                                            ClaI
                                          ~~~~~~
1001 TTGATTCTGT CGCTACTGAT TACGGTGCTG CTATCGATGG TTTCATTGGT
     AACTAAGACA GCGATGACTA ATGCCACGAC GATAGCTACC AAAGTAACCA

1051 GACGTTTCCG GCCTTGCTAA TGGTAATGGT GCTACTGGTG ATTTTGCTGG
     CTGCAAAGGC CGGAACGATT ACCATTACCA CGATGACCAC TAAAACGACC
```

```
1101   CTCTAATTCC CAAATGGCTC AAGTCGGTGA CGGTGATAAT TCACCTTTAA
       GAGATTAAGG GTTTACCGAG TTCAGCCACT GCCACTATTA AGTGGAAATT

1151   TGAATAATTT CCGTCAATAT TTACCTTCCC TCCCTCAATC GGTTGAATGT
       ACTTATTAAA GGCAGTTATA AATGGAAGGG AGGGAGTTAG CCAACTTACA

1201   CGCCCTTTTG TCTTTGGCGC TGGTAAACCA TATGAATTTT CTATTGATTG
       GCGGGAAAAC AGAAACCGCG ACCATTTGGT ATACTTAAAA GATAACTAAC

1251   TGACAAAATA AACTTATTCC GTGGTGTCTT TGCGTTTCTT TTATATGTTG
       ACTGTTTTAT TTGAATAAGG CACCACAGAA ACGCAAAGAA AATATACAAC

1301   CCACCTTTAT GTATGTATTT TCTACGTTTG CTAACATACT GCGTAATAAG
       GGTGGAAATA CATACATAAA AGATGCAAAC GATTGTATGA CGCATTATTC

                  HindIII
                  ~~~~~~
1351   GAGTCTTGAT AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG
       CTCAGAACTA TTCGAACTGG ACACTTCACT TTTTACCGCG TCTAACACGC
                                OGIII3  100.0%
                          ====================

1401   ACATTTTTTT TGTCTGCCGT TTAATGAAAT TGTAAACGTT AATATTTTGT
       TGTAAAAAAA ACAGACGGCA AATTACTTTA ACATTTGCAA TTATAAAACA

1451   TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT TAACCAATAG
       ATTTTAAGCG CAATTTAAAA ACAATTTAGT CGAGTAAAAA ATTGGTTATC

1501   GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG
       CGGCTTTAGC CGTTTTAGGG AATATTTAGT TTTCTTATCT GGCTCTATCC

1551   GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG
       CAACTCACAA CAAGGTCAAA CCTTGTTCTC AGGTGATAAT TTCTTGCACC

1601   ACTCCAACGT CAAAGGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA
       TGAGGTTGCA GTTTCCCGCT TTTTGGCAGA TAGTCCCGCT ACCGGGTGAT

1651   CGAGAACCAT CACCCTAATC AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC
       GCTCTTGGTA GTGGGATTAG TTCAAAAAAC CCCAGCTCCA CGGCATTTCG

1701   ACTAAATCGG AACCCTAAAG GGAGCCCCCG ATTTAGAGCT TGACGGGGAA
       TGATTTAGCC TTGGGATTTC CCTCGGGGGC TAAATCTCGA ACTGCCCCTT

1751   AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA AGGAGCGGGC
       TCGGCCGCTT GCACCGCTCT TTCCTTCCCT TCTTTCGCTT TCCTCGCCCG

1801   GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA CCACCACACC
       CGATCCCGCG ACCGTTCACA TCGCCAGTGC GACGCGCATT GGTGGTGTGG

1851   CGCCGCGCTT AATGCGCCGC TACAGGGCGC GTGCTAGCCA TGTGAGCAAA
       GCGGCGCGAA TTACGCGGCG ATGTCCCGCG CACGATCGGT ACACTCGTTT

1901   AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT
       TCCGGTCGTT TTCCGGTCCT TGGCATTTTT CCGGCGCAAC GACCGCAAAA

1951   TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT
       AGGTATCCGA GGCGGGGGGA CTGCTCGTAG TGTTTTTAGC TGCGAGTTCA
```

```
2001  CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC
      GTCTCCACCG CTTTGGGCTG TCCTGATATT TCTATGGTCC GCAAAGGGGG

2051  TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT
      ACCTTCGAGG GAGCACGCGA GAGGACAAGG CTGGGACGGC GAATGGCCTA

2101  ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCATAGCTCA
      TGGACAGGCG GAAAGAGGGA AGCCCTTCGC ACCGCGAAAG AGTATCGAGT

2151  CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG
      GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT TCGACCCGAC

        ApaLI
       ~~~~~~
2201  TGTGCACGAA CCCCCCGTTC AGTCCGACCG CTGCGCCTTA TCCGGTAACT
      ACACGTGCTT GGGGGGCAAG TCAGGCTGGC GACGCGGAAT AGGCCATTGA

2251  ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
      TAGCAGAACT CAGGTTGGGC CATTCTGTGC TGAATAGCGG TGACCGTCGT

2301  GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA
      CGGTGACCAT TGTCCTAATC GTCTCGCTCC ATACATCCGC CACGATGTCT

2351  GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGA ACAGTATTTG
      CAAGAACTTC ACCACCGGAT TGATGCCGAT GTGATCTTCT TGTCATAAAC

2401  GTATCTGCGC TCTGCTGTAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC
      CATAGACGCG AGACGACATC GGTCAATGGA AGCCTTTTTC TCAACCATCG

2451  TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG
      AGAACTAGGC CGTTTGTTTG GTGGCGACCA TCGCCACCAA AAAAACAAAC

2501  CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA
      GTTCGTCGTC TAATGCGCGT CTTTTTTTCC TAGAGTTCTT CTAGGAAACT

2551  TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
      AGAAAAGATG CCCCAGACTG CGAGTCACCT TGCTTTTGAG TGCAATTCCC

2601  ATTTTGGTCA GATCTAGCAC CAGGCGTTTA AGGGCACCAA TAACTGCCTT
      TAAAACCAGT CTAGATCGTG GTCCGCAAAT TCCCGTGGTT ATTGACGGAA

2651  AAAAAAATTA CGCCCCGCCC TGCCACTCAT CGCAGTACTG TTGTAATTCA
      TTTTTTTAAT GCGGGGCGGG ACGGTGAGTA GCGTCATGAC AACATTAAGT

2701  TTAAGCATTC TGCCGACATG GAAGCCATCA CAAACGGCAT GATGAACCTG
      AATTCGTAAG ACGGCTGTAC CTTCGGTAGT GTTTGCCGTA CTACTTGGAC

2751  AATCGCCAGC GGCATCAGCA CCTTGTCGCC TTGCGTATAA TATTTGCCCA
      TTAGCGGTCG CCGTAGTCGT GGAACAGCGG AACGCATATT ATAAACGGGT

2801  TAGTGAAAAC GGGGGCGAAG AAGTTGTCCA TATTGGCTAC GTTTAAATCA
      ATCACTTTTG CCCCCGCTTC TTCAACAGGT ATAACCGATG CAAATTTAGT

2851  AAACTGGTGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC
      TTTGACCACT TTGAGTGGGT CCCTAACCGA CTCTGCTTTT TGTATAAGAG
```

```
2901  AATAAACCCT TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT
      TTATTTGGGA AATCCCTTTA TCCGGTCCAA AAGTGGCATT GTGCGGTGTA

2951  CTTGCGAATA TATGTGTAGA AACTGCCGGA AATCGTCGTG GTATTCACTC
      GAACGCTTAT ATACACATCT TTGACGGCCT TTAGCAGCAC CATAAGTGAG

3001  CAGAGCGATG AAAACGTTTC AGTTTGCTCA TGGAAAACGG TGTAACAAGG
      GTCTCGCTAC TTTTGCAAAG TCAAACGAGT ACCTTTTGCC ACATTGTTCC

3051  GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT GCCATACGGA
      CACTTGTGAT AGGGTATAGT GGTCGAGTGG CAGAAAGTAA CGGTATGCCT

3101  ACTCCGGGTG AGCATTCATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA
      TGAGGCCCAC TCGTAAGTAG TCCGCCCGTT CTTACACTTA TTTCCGGCCT

3151  TAAAACTTGT GCTTATTTTT CTTTACGGTC TTTAAAAAGG CCGTAATATC
      ATTTTGAACA CGAATAAAAA GAAATGCCAG AAATTTTTCC GGCATTATAG

3201  CAGCTGAACG GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT
      GTCGACTTGC CAGACCAATA TCCATGTAAC TCGTTGACTG ACTTTACGGA

3251  CAAAATGTTC TTTACGATGC CATTGGGATA TATCAACGGT GGTATATCCA
      GTTTTACAAG AAATGCTACG GTAACCCTAT ATAGTTGCCA CCATATAGGT

3301  GTGATTTTTT TCTCCATTTT AGCTTCCTTA GCTCCTGAAA ATCTCGATAA
      CACTAAAAAA AGAGGTAAAA TCGAAGGAAT CGAGGACTTT TAGAGCTATT

3351  CTCAAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG TGAAAGTTGG
      GAGTTTTTTA TGCGGGCCAT CACTAGAATA AAGTAATACC ACTTTCAACC

3401  AACCTCACCC GACGTCTAAT GTGAGTTAGC TCACTCATTA GGCACCCCAG
      TTGGAGTGGG CTGCAGATTA CACTCAATCG AGTGAGTAAT CCGTGGGGTC

3451  GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG
      CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT AACACTCGCC

                 M13 Reverse  primer 100.0%           XbaI
                     ===================               ~~
3501  ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTTCT
      TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAAAGA
```

Figure 12

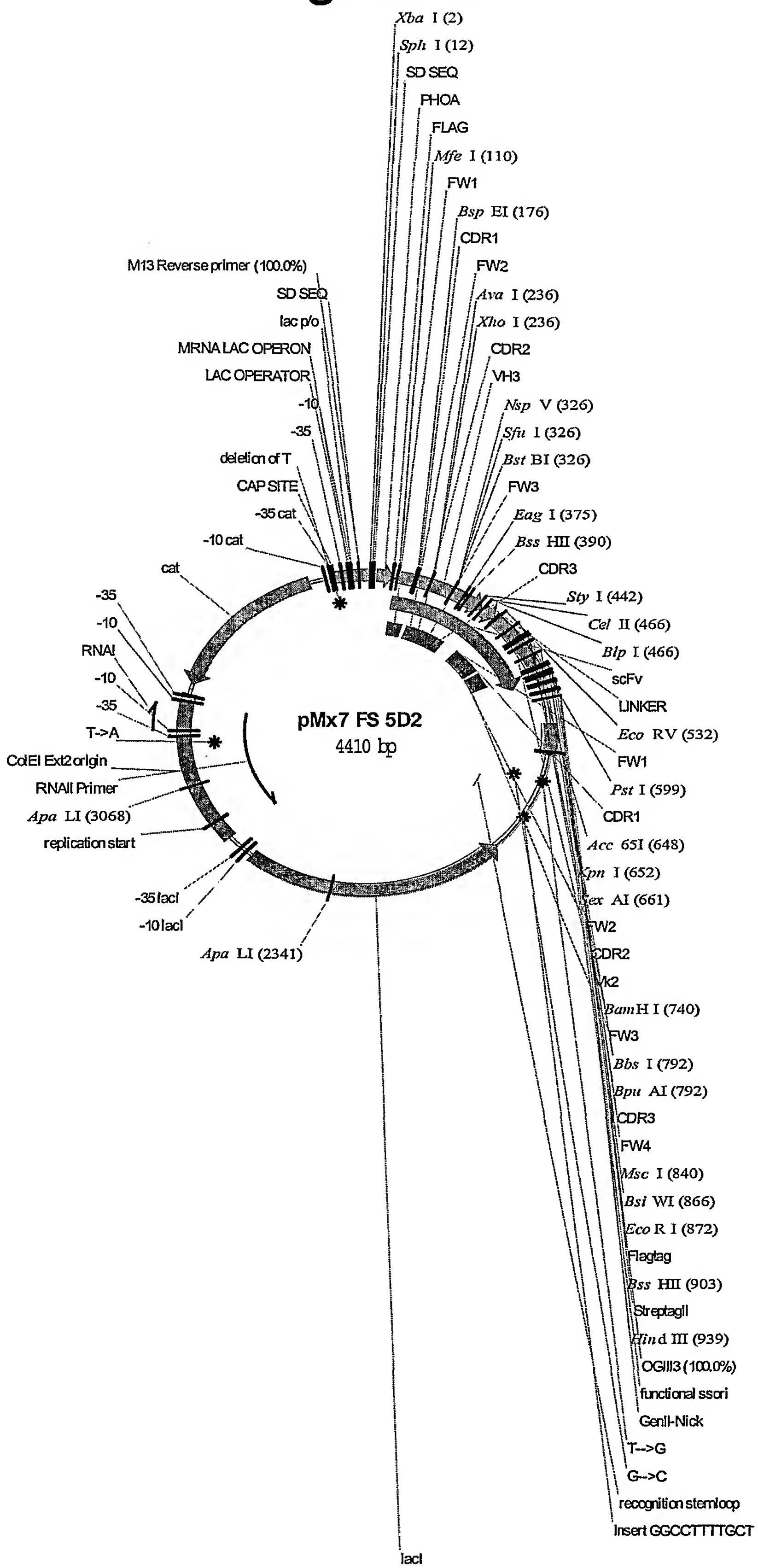

pMx7 FS 5D2
4410 bp
Xba I (2)
Sph I (12)
SD SEQ
PHOA
FLAG
Mfe I (110)
FW1
Bsp EI (176)
CDR1
FW2
Ava I (236)
Xho I (236)
CDR2
VH3
Nsp V (326)
Sfu I (326)
Bst BI (326)
FW3
Eag I (375)
Bss HII (390)
CDR3
Sty I (442)
Cel II (466)
Blp I (466)
scFv
LINKER
Eco RV (532)
FW1
Pst I (599)
CDR1
Acc 65I (648)
Kpn I (652)
Sex AI (661)
FW2
CDR2
Vk2
BamH I (740)
FW3
Bbs I (792)
Bpu AI (792)
CDR3
FW4
Msc I (840)
Bsi WI (866)
Eco R I (872)
Flagtag
Bss HII (903)
StreptagII
Hind III (939)
OGIII3 (100.0%)
functional ssori
GenII-Nick
T-->G
G-->C
recognition stemloop
Insert GGCCTTTTGCT
lacI
Apa LI (2341)
-10 lacI
-35 lacI
replication start
Apa LI (3068)
RNAII Primer
ColEI Ext2 origin
T->A
-35
-10
RNAI
-10
-35
cat
-10 cat
-35 cat
CAP SITE
deletion of T
-35
-10
LAC OPERATOR
MRNA LAC OPERON
lac p/o
SD SEQ
M13 Reverse primer (100.0%)

Figure 12 (cont)

```
      XbaI   SphI
     ~~~~~~~~~~~~~~
  1  TCTAGAGCAT GCGTAGGAGA AAATAAAATG AAACAAAGCA CTATTGCACT
     AGATCTCGTA CGCATCCTCT TTTATTTTAC TTTGTTTCGT GATAACGTGA

 51  GGCACTCTTA CCGTTGCTCT TCACCCCTGT TACCAAAGCC GACTACAAAG
     CCGTGAGAAT GGCAACGAGA AGTGGGGACA ATGGTTTCGG CTGATGTTTC

              MfeI
             ~~~~~~~
101  ATGAAGTGCA ATTGGTGGAA AGCGGCGGCG GCCTGGTGCA ACCGGGCGGC
     TACTTCACGT TAACCACCTT TCGCCGCCGC CGGACCACGT TGGCCCGCCG

                             BspEI
                            ~~~~~~
151  AGCCTGCGTC TGAGCTGCGC GGCCTCCGGA TTTACCTTTA GCAGCTATGC
     TCGGACGCAG ACTCGACGCG CCGGAGGCCT AAATGGAAAT CGTCGATACG

                                         XhoI
                                        ~~~~~~
                                         AvaI
                                        ~~~~~~
201  GATGAGCTGG GTGCGCCAAG CCCCTGGGAA GGGTCTCGAG TGGGTGAGCG
     CTACTCGACC CACGCGGTTC GGGGACCCTT CCCAGAGCTC ACCCACTCGC

251  CGATTAGCGG TAGCGGCGGC AGCACCTATT ATGCGGATAG CGTGAAAGGC
     GCTAATCGCC ATCGCCGCCG TCGTGGATAA TACGCCTATC GCACTTTCCG

                            BstBI
                           ~~~~~~
                            SfuI
                           ~~~~~~
                            NspV
                           ~~~~~~
301  CGTTTTACCA TTTCACGTGA TAATTCGAAA AACACCCTGT ATCTGCAAAT
     GCAAAATGGT AAAGTGCACT ATTAAGCTTT TTGTGGGACA TAGACGTTTA

                            EagI          BssHII
                           ~~~~~~         ~~~~~~~
351  GAACAGCCTG CGTGCGGAAG ATACGGCCGT GTATTATTGC GCGCGTGTTA
     CTTGTCGGAC GCACGCCTTC TATGCCGGCA CATAATAACG CGCGCACAAT

                                                  StyI
                                                 ~~~~~~
401  AGAAGCATTT TTCTCGTAAG AATTGGTTTG ATTATTGGGG CCAAGGCACC
     TCTTCGTAAA AAGAGCATTC TTAACCAAAC TAATAACCCC GGTTCCGTGG
```

```
                    BlpI
                   ~~~~~~~~
                    CelII
                   ~~~~~~~~
451   CTGGTGACGG TTAGCTCAGC GGGTGGCGGT TCTGGCGGCG GTGGGAGCGG
      GACCACTGCC AATCGAGTCG CCCACCGCCA AGACCGCCGC CACCCTCGCC

                                   EcoRV
                                  ~~~~~~~~
501   TGGCGGTGGT TCTGGCGGTG GTGGTTCCGA TATCGTGATG ACCCAGAGCC
      ACCGCCACCA AGACCGCCAC CACCAAGGCT ATAGCACTAC TGGGTCTCGG

                                                    PstI
                                                   ~~~~~~~
551   CACTGAGCCT GCCAGTGACT CCGGGCGAGC CTGCGAGCAT TAGCTGCAGA
      GTGACTCGGA CGGTCACTGA GGCCCGCTCG GACGCTCGTA ATCGACGTCT

                                                      KpnI
                                                      ~~~~
                                                    Acc65I
                                                      ~~~~
601   AGCAGCCAAA GCCTGCTGCA TAGCAACGGC TATAACTATC TGGATTGGTA
      TCGTCGGTTT CGGACGACGT ATCGTTGCCG ATATTGATAG ACCTAACCAT

      KpnI
      ~~
      Acc65I      SexAI
      ~~        ~~~~~~~~~
651   CCTTTCAAAA CCAGGTCAAA GCCCGCAGCT ATTAATTTAT CTGGGCAGCA
      GGAAGTTTTT GGTCCAGTTT CGGGCGTCGA TAATTAAATA GACCCGTCGT

                                              BamHI
                                             ~~~~~~~~
701   ACCGTGCCAG TGGGGTCCCG GATCGTTTTA GCGGCTCTGG ATCCGGCACC
      TGGCACGGTC ACCCCAGGGC CTAGCAAAAT CGCCGAGACC TAGGCCGTGG

                                         BpuAI
                                        ~~~~~~~
                                         BbsI
                                        ~~~~~~~
751   GATTTTACCC TGAAAATTAG CCGTGTGGAA GCTGAAGACG TGGGCGTGTA
      CTAAAATGGG ACTTTTAATC GGCACACCTT CGACTTCTGC ACCCGCACAT

                                            MscI
                                           ~~~~~~~~
801   TTATTGCCAG CAGCATTATA CCACCCCGCC GACCTTTGGC CAGGGTACGA
      AATAACGGTC GTCGTAATAT GGTGGGGCGG CTGGAAACCG GTCCCATGCT
```

```
                    BsiWI   EcoRI
                   ~~~~~~~~~~~~~~
 851  AAGTTGAAAT TAAACGTACG GAATTCGACT ATAAAGATGA CGATGACAAA
      TTCAACTTTA ATTTGCATGC CTTAAGCTGA TATTTCTACT GCTACTGTTT

       BssHII                                   HindIII
       ~~~~~~                                   ~~~~~~~
 901  GGCGCGCCGT GGAGCCACCC GCAGTTTGAA AAATGATAAG CTTGACCTGT
      CCGCGCGGCA CCTCGGTGGG CGTCAAACTT TTTACTATTC GAACTGGACA
                                                OGIII3  100.0%
                                                       =======

 951  GAAGTGAAAA ATGGCGCAGA TTGTGCGACA TTTTTTTTGT CTGCCGTTTA
      CTTCACTTTT TACCGCGTCT AACACGCTGT AAAAAAAACA GACGGCAAAT
      OGIII3 100.0%
      ============

1001  ATTAAAGGGG GGGGGGGGCC GGCCTGGGGG GGGGTGTACA TGAAATTGTA
      TAATTTCCCC CCCCCCCCGG CCGGACCCCC CCCCACATGT ACTTTAACAT

1051  AACGTTAATA TTTTGTTAAA ATTCGCGTTA AATTTTTGTT AAATCAGCTC
      TTGCAATTAT AAAACAATTT TAAGCGCAAT TTAAAAACAA TTTAGTCGAG

1101  ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG
      TAAAAAATTG GTTATCCGGC TTTAGCCGTT TTAGGGAATA TTTAGTTTTC

1151  AATAGACCGA GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA
      TTATCTGGCT CTATCCCAAC TCACAACAAG GTCAAACCTT GTTCTCAGGT

1201  CTATTAAAGA ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA
      GATAATTTCT TGCACCTGAG GTTGCAGTTT CCCGCTTTTT GGCAGATAGT

1251  GGGCGATGGC CCACTACGAG AACCATCACC CTAATCAAGT TTTTTGGGGT
      CCCGCTACCG GGTGATGCTC TTGGTAGTGG GATTAGTTCA AAAAACCCCA

1301  CGAGGTGCCG TAAAGCACTA AATCGGAACC CTAAAGGGAG CCCCCGATTT
      GCTCCACGGC ATTTCGTGAT TTAGCCTTGG GATTTCCCTC GGGGGCTAAA

1351  AGAGCTTGAC GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA
      TCTCGAACTG CCCCTTTCGG CCGCTTGCAC CGCTCTTTCC TTCCCTTCTT

1401  AGCGAAAGGA GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC
      TCGCTTTCCT CGCCCGCGAT CCCGCGACCG TTCACATCGC CAGTGCGACG

1451  GCGTAACCAC CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTGC
      CGCATTGGTG GTGTGGGCGG CGCGAATTAC GCGGCGATGT CCCGCGCACG
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1501  TAGACTAGTG TTTAAACCGG ACCGGGGGGG GGCTTAAGTG GGCTGCAAAA
      ATCTGATCAC AAATTTGGCC TGGCCCCCCC CCGAATTCAC CCGACGTTTT

1551  CAAAACGGCC TCCTGTCAGG AAGCCGCTTT TATCGGGTAG CCTCACTGCC
      GTTTTGCCGG AGGACAGTCC TTCGGCGAAA ATAGCCCATC GGAGTGACGG

1601  CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGCATCAG TGAATCGGCC
      GCGAAAGGTC AGCCCTTTGG ACAGCACGGT CGACGTAGTC ACTTAGCCGG

1651  AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGAGCCAGGG TGGTTTTTCT
      TTGCGCGCCC CTCTCCGCCA AACGCATAAC CCTCGGTCCC ACCAAAAAGA

1701  TTTCACCAGT GAGACGGGCA ACAGCTGATT GCCCTTCACC GCCTGGCCCT
      AAAGTGGTCA CTCTGCCCGT TGTCGACTAA CGGGAAGTGG CGGACCGGGA

1751  GAGAGAGTTG CAGCAAGCGG TCCACGCTGG TTTGCCCCAG CAGGCGAAAA
      CTCTCTCAAC GTCGTTCGCC AGGTGCGACC AAACGGGGTC GTCCGCTTTT

1801  TCCTGTTTGA TGGTGGTCAG CGGCGGGATA TAACATGAGC TGTCCTCGGT
      AGGACAAACT ACCACCAGTC GCCGCCCTAT ATTGTACTCG ACAGGAGCCA

1851  ATCGTCGTAT CCCACTACCG AGATGTCCGC ACCAACGCGC AGCCCGGACT
      TAGCAGCATA GGGTGATGGC TCTACAGGCG TGGTTGCGCG TCGGGCCTGA

1901  CGGTAATGGC ACGCATTGCG CCCAGCGCCA TCTGATCGTT GGCAACCAGC
      GCCATTACCG TGCGTAACGC GGGTCGCGGT AGACTAGCAA CCGTTGGTCG

1951  ATCGCAGTGG GAACGATGCC CTCATTCAGC ATTTGCATGG TTTGTTGAAA
      TAGCGTCACC CTTGCTACGG GAGTAAGTCG TAAACGTACC AAACAACTTT

2001  ACCGGACATG GCACTCCAGT CGCCTTCCCG TTCCGCTATC GGCTGAATTT
      TGGCCTGTAC CGTGAGGTCA GCGGAAGGGC AAGGCGATAG CCGACTTAAA

2051  GATTGCGAGT GAGATATTTA TGCCAGCCAG CCAGACGCAG ACGCGCCGAG
      CTAACGCTCA CTCTATAAAT ACGGTCGGTC GGTCTGCGTC TGCGCGGCTC

2101  ACAGAACTTA ATGGGCCAGC TAACAGCGCG ATTTGCTGGT GGCCCAATGC
      TGTCTTGAAT TACCCGGTCG ATTGTCGCGC TAAACGACCA CCGGGTTACG

2151  GACCAGATGC TCCACGCCCA GTCGCGTACC GTCCTCATGG GAGAAAATAA
      CTGGTCTACG AGGTGCGGGT CAGCGCATGG CAGGAGTACC CTCTTTTATT

2201  TACTGTTGAT GGGTGTCTGG TCAGAGACAT CAAGAAATAA CGCCGGAACA
      ATGACAACTA CCCACAGACC AGTCTCTGTA GTTCTTTATT GCGGCCTTGT

2251  TTAGTGCAGG CAGCTTCCAC AGCAATAGCA TCCTGGTCAT CCAGCGGATA
      AATCACGTCC GTCGAAGGTG TCGTTATCGT AGGACCAGTA GGTCGCCTAT

                                                ApaLI
                                               ~~~~~~~
2301  GTTAATAATC AGCCCACTGA CACGTTGCGC GAGAAGATTG TGCACCGCCG
      CAATTATTAG TCGGGTGACT GTGCAACGCG CTCTTCTAAC ACGTGGCGGC

2351  CTTTACAGGC TTCGACGCCG CTTCGTTCTA CCATCGACAC GACCACGCTG
      GAAATGTCCG AAGCTGCGGC GAAGCAAGAT GGTAGCTGTG CTGGTGCGAC
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2401  GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA TTTGCGACGG
      CGTGGGTCAA CTAGCCGCGC TCTAAATTAG CGGCGCTGTT AAACGCTGCC

2451  CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT
      GCGCACGTCC CGGTCTGACC TCCACCGTTG CGGTTAGTCG TTGCTGACAA

2501  TGCCCGCCAG TTGTTGTGCC ACGCGGTTAG GAATGTAATT CAGCTCCGCC
      ACGGGCGGTC AACAACACGG TGCGCCAATC CTTACATTAA GTCGAGGCGG

2551  ATCGCCGCTT CCACTTTTTC CCGCGTTTTC GCAGAAACGT GGCTGGCCTG
      TAGCGGCGAA GGTGAAAAAG GGCGCAAAAG CGTCTTTGCA CCGACCGGAC

2601  GTTCACCACG CGGGAAACGG TCTGATAAGA GACACCGGCA TACTCTGCGA
      CAAGTGGTGC GCCCTTTGCC AGACTATTCT CTGTGGCCGT ATGAGACGCT

2651  CATCGTATAA CGTTACTGGT TTCACATTCA CCACCCTGAA TTGACTCTCT
      GTAGCATATT GCAATGACCA AAGTGTAAGT GGTGGGACTT AACTGAGAGA

2701  TCCGGGCGCT ATCATGCCAT ACCGCGAAAG GTTTTGCGCC ATTCGATGCT
      AGGCCCGCGA TAGTACGGTA TGGCGCTTTC CAAAACGCGG TAAGCTACGA

2751  AGCCATGTGA GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG
      TCGGTACACT CGTTTTCCGG TCGTTTTCCG GTCCTTGGCA TTTTTCCGGC

2801  CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA GCATCACAAA
      GCAACGACCG CAAAAAGGTA TCCGAGGCGG GGGGACTGCT CGTAGTGTTT

2851  AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA
      TTAGCTGCGA GTTCAGTCTC CACCGCTTTG GGCTGTCCTG ATATTTCTAT

2901  CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC
      GGTCCGCAAA GGGGGACCTT CGAGGGAGCA CGCGAGAGGA CAAGGCTGGG

2951  TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG
      ACGGCGAATG GCCTATGGAC AGGCGGAAAG AGGGAAGCCC TTCGCACCGC

3001  CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTCG
      GAAAGAGTAT CGAGTGCGAC ATCCATAGAG TCAAGCCACA TCCAGCAAGC

                             ApaLI
                            ~~~~~~~
3051  CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG
      GAGGTTCGAC CCGACACACG TGCTTGGGGG GCAAGTCGGG CTGGCGACGC

3101  CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA
      GGAATAGGCC ATTGATAGCA GAACTCAGGT TGGGCCATTC TGTGCTGAAT

3151  TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT
      AGCGGTGACC GTCGTCGGTG ACCATTGTCC TAATCGTCTC GCTCCATACA

3201  AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA
      TCCGCCACGA TGTCTCAAGA ACTTCACCAC CGGATTGATG CCGATGTGAT

3251  GAAGAACAGT ATTTGGTATC TGCGCTCTGC TGTAGCCAGT TACCTTCGGA
      CTTCTTGTCA TAAACCATAG ACGCGAGACG ACATCGGTCA ATGGAAGCCT
```

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3301  AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG CTGGTAGCGG
      TTTTCTCAAC CATCGAGAAC TAGGCCGTTT GTTTGGTGGC GACCATCGCC

3351  TGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC
      ACCAAAAAAA CAAACGTTCG TCGTCTAATG CGCGTCTTTT TTTCCTAGAG

3401  AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA
      TTCTTCTAGG AAACTAGAAA AGATGCCCCA GACTGCGAGT CACCTTGCTT

3451  AACTCACGTT AAGGGATTTT GGTCAGATCT AGCACCAGGC GTTTAAGGGC
      TTGAGTGCAA TTCCCTAAAA CCAGTCTAGA TCGTGGTCCG CAAATTCCCG

3501  ACCAATAACT GCCTTAAAAA AATTACGCCC CGCCCTGCCA CTCATCGCAG
      TGGTTATTGA CGGAATTTTT TTAATGCGGG GCGGGACGGT GAGTAGCGTC

3551  TACTGTTGTA ATTCATTAAG CATTCTGCCG ACATGGAAGC CATCACAAAC
      ATGACAACAT TAAGTAATTC GTAAGACGGC TGTACCTTCG GTAGTGTTTG

3601  GGCATGATGA ACCTGAATCG CCAGCGGCAT CAGCACCTTG TCGCCTTGCG
      CCGTACTACT TGGACTTAGC GGTCGCCGTA GTCGTGGAAC AGCGGAACGC

3651  TATAATATTT GCCCATAGTG AAAACGGGGG CGAAGAAGTT GTCCATATTG
      ATATTATAAA CGGGTATCAC TTTTGCCCCC GCTTCTTCAA CAGGTATAAC

3701  GCTACGTTTA AATCAAAACT GGTGAAACTC ACCCAGGGAT TGGCTGAGAC
      CGATGCAAAT TTAGTTTTGA CCACTTTGAG TGGGTCCCTA ACCGACTCTG

3751  GAAAAACATA TTCTCAATAA ACCCTTTAGG GAAATAGGCC AGGTTTTCAC
      CTTTTTGTAT AAGAGTTATT TGGGAAATCC CTTTATCCGG TCCAAAAGTG

3801  CGTAACACGC CACATCTTGC GAATATATGT GTAGAAACTG CCGGAAATCG
      GCATTGTGCG GTGTAGAACG CTTATATACA CATCTTTGAC GGCCTTTAGC

3851  TCGTGGTATT CACTCCAGAG CGATGAAAAC GTTTCAGTTT GCTCATGGAA
      AGCACCATAA GTGAGGTCTC GCTACTTTTG CAAAGTCAAA CGAGTACCTT

3901  AACGGTGTAA CAAGGGTGAA CACTATCCCA TATCACCAGC TCACCGTCTT
      TTGCCACATT GTTCCCACTT GTGATAGGGT ATAGTGGTCG AGTGGCAGAA

3951  TCATTGCCAT ACGGAACTCC GGGTGAGCAT TCATCAGGCG GGCAAGAATG
      AGTAACGGTA TGCCTTGAGG CCCACTCGTA AGTAGTCCGC CCGTTCTTAC

4001  TGAATAAAGG CCGGATAAAA CTTGTGCTTA TTTTTCTTTA CGGTCTTTAA
      ACTTATTTCC GGCCTATTTT GAACACGAAT AAAAAGAAAT GCCAGAAATT

4051  AAAGGCCGTA ATATCCAGCT GAACGGTCTG GTTATAGGTA CATTGAGCAA
      TTTCCGGCAT TATAGGTCGA CTTGCCAGAC CAATATCCAT GTAACTCGTT

4101  CTGACTGAAA TGCCTCAAAA TGTTCTTTAC GATGCCATTG GGATATATCA
      GACTGACTTT ACGGAGTTTT ACAAGAAATG CTACGGTAAC CCTATATAGT

4151  ACGGTGGTAT ATCCAGTGAT TTTTTTCTCC ATTTTAGCTT CCTTAGCTCC
      TGCCACCATA TAGGTCACTA AAAAAAGAGG TAAAATCGAA GGAATCGAGG

4201  TGAAAATCTC GATAACTCAA AAAATACGCC CGGTAGTGAT CTTATTTCAT
      ACTTTTAGAG CTATTGAGTT TTTTATGCGG GCCATCACTA GAATAAAGTA
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4251  TATGGTGAAA GTTGGAACCT CACCCGACGT CTAATGTGAG TTAGCTCACT
      ATACCACTTT CAACCTTGGA GTGGGCTGCA GATTACACTC AATCGAGTGA

4301  CATTAGGCAC CCCAGGCTTT ACACTTTATG CTTCCGGCTC GTATGTTGTG
      GTAATCCGTG GGGTCCGAAA TGTGAAATAC GAAGGCCGAG CATACAACAC

                                   M13 Reverse  primer  100.0%
                                      ===================
4351  TGGAATTGTG AGCGGATAAC AATTTCACAC AGGAAACAGC TATGACCATG
      ACCTTAACAC TCGCCTATTG TTAAAGTGTG TCCTTTGTCG ATACTGGTAC

4401  ATTACGAATT
      TAATGCTTAA
```

Figure 13

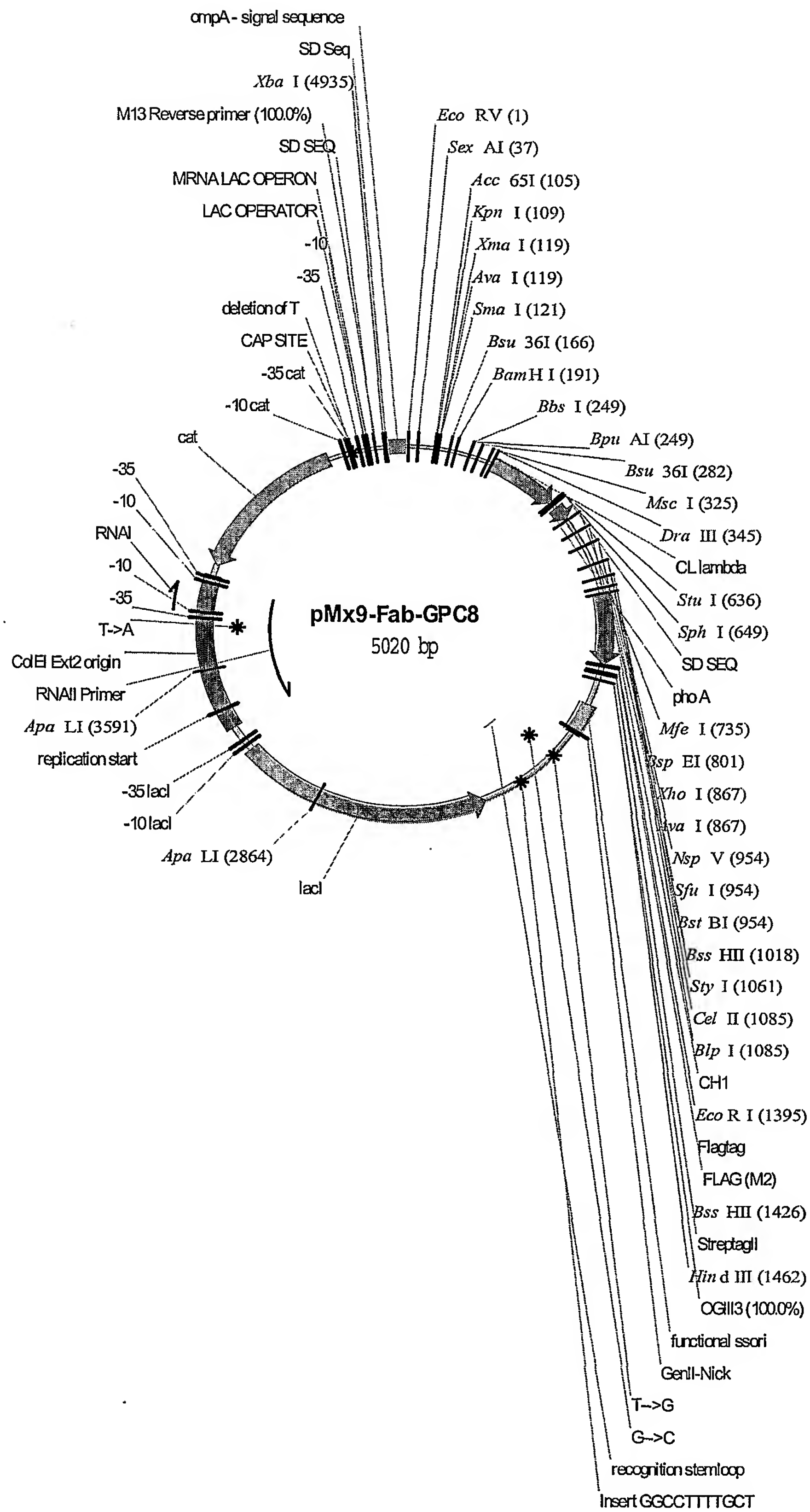
ompA - signal sequence
SD Seq
Xba I (4935)
M13 Reverse primer (100.0%)
SD SEQ
MRNA LAC OPERON
LAC OPERATOR
-10
-35
deletion of T
CAP SITE
-35 cat
-10 cat
cat
-35
-10
RNAI
-10
-35
T->A
ColEI Ext2 origin
RNAII Primer
Apa LI (3591)
replication start
-35 lacI
-10 lacI
Apa LI (2864)
lacI
Eco RV (1)
Sex AI (37)
Acc 65I (105)
Kpn I (109)
Xma I (119)
Ava I (119)
Sma I (121)
Bsu 36I (166)
BamH I (191)
Bbs I (249)
Bpu AI (249)
Bsu 36I (282)
Msc I (325)
Dra III (345)
CL lambda
Stu I (636)
Sph I (649)
SD SEQ
pho A
Mfe I (735)
Bsp EI (801)
Xho I (867)
Ava I (867)
Nsp V (954)
Sfu I (954)
Bst BI (954)
Bss HII (1018)
Sty I (1061)
Cel II (1085)
Blp I (1085)
CH1
Eco R I (1395)
Flagtag
FLAG (M2)
Bss HII (1426)
StreptagII
Hind III (1462)
OGIII3 (100.0%)
functional ssori
GenII-Nick
T-->G
G-->C
recognition stemloop
Insert GGCCTTTTGCT
pMx9-Fab-GPC8
5020 bp

Figure 13 (cont)

```
     EcoRV                                                SexAI
     ~~~                                                ~~~~~~~~~
  1  ATCGTGCTGA CCCAGCCGCC TTCAGTGAGT GGCGCACCAG GTCAGCGTGT
     TAGCACGACT GGGTCGGCGG AAGTCACTCA CCGCGTGGTC CAGTCGCACA

 51  GACCATCTCG TGTAGCGGCA GCAGCAGCAA CATTGGCAGC AACTATGTGA
     CTGGTAGAGC ACATCGCCGT CGTCGTCGTT GTAACCGTCG TTGATACACT

                           XmaI
                         ~~~~~~~~
        KpnI               SmaI
       ~~~~~~            ~~~~~~~~
       Acc65I              AvaI
       ~~~~~~            ~~~~~~~~
101  GCTGGTACCA GCAGTTGCCC GGGACGGCGC CGAAACTGCT GATTTATGAT
     CGACCATGGT CGTCAACGGG CCCTGCCGCG GCTTTGACGA CTAAATACTA

                    Bsu36I                              BamHI
                   ~~~~~~~                            ~~~~~~~
151  AACAACCAGC GTCCCTCAGG CGTGCCGGAT CGTTTTAGCG GATCCAAAAG
     TTGTTGGTCG CAGGGAGTCC GCACGGCCTA GCAAAATCGC CTAGGTTTTC

                                                        BpuAI
                                                       ~~~~~~
                                                        BbsI
                                                       ~~~~~~
201  CGGCACCAGC GCGAGCCTTG CGATTACGGG CCTGCAAAGC GAAGACGAAG
     GCCGTGGTCG CGCTCGGAAC GCTAATGCCC GGACGTTTCG CTTCTGCTTC

                                       Bsu36I
                                      ~~~~~~~~
251  CGGATTATTA TTGCCAGAGC TATGACATGC CTCAGGCTGT GTTTGGCGGC
     GCCTAATAAT AACGGTCTCG ATACTGTACG GAGTCCGACA CAAACCGCCG

                              MscI              DraIII
                             ~~~~~~           ~~~~~~~~~~
301  GGCACGAAGT TTAACCGTTC TTGGCCAGCC GAAAGCCGCA CCGAGTGTGA
     CCGTGCTTCA AATTGGCAAG AACCGGTCGG CTTTCGGCGT GGCTCACACT

351  CGCTGTTTCC GCCGAGCAGC GAAGAATTGC AGGCGAACAA AGCGACCCTG
     GCGACAAAGG CGGCTCGTCG CTTCTTAACG TCCGCTTGTT TCGCTGGGAC

401  GTGTGCCTGA TTAGCGACTT TTATCCGGGA GCCGTGACAG TGGCCTGGAA
     CACACGGACT AATCGCTGAA AATAGGCCCT CGGCACTGTC ACCGGACCTT

451  GGCAGATAGC AGCCCCGTCA AGGCGGGAGT GGAGACCACC ACACCCTCCA
     CCGTCTATCG TCGGGGCAGT TCCGCCCTCA CCTCTGGTGG TGTGGGAGGT

501  AACAAAGCAA CAACAAGTAC GCGGCCAGCA GCTATCTGAG CCTGACGCCT
     TTGTTTCGTT GTTGTTCATG CGCCGGTCGT CGATAGACTC GGACTGCGGA

551  GAGCAGTGGA AGTCCCACAG AAGCTACAGC TGCCAGGTCA CGCATGAGGG
     CTCGTCACCT TCAGGGTGTC TTCGATGTCG ACGGTCCAGT GCGTACTCCC

                                          StuI        SphI
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                                          ~~~~~~      ~~~~~~
 601  GAGCACCGTG GAAAAAACCG TTGCGCCGAC TGAGGCCTGA TAAGCATGCG
      CTCGTGGCAC CTTTTTTGGC AACGCGGCTG ACTCCGGACT ATTCGTACGC

 651  TAGGAGAAAA TAAAATGAAA CAAAGCACTA TTGCACTGGC ACTCTTACCG
      ATCCTCTTTT ATTTTACTTT GTTTCGTGAT AACGTGACCG TGAGAATGGC

                                           MfeI
                                          ~~~~~~
 701  TTGCTCTTCA CCCCTGTTAC CAAAGCCCAG GTGCAATTGA AAGAAAGCGG
      AACGAGAAGT GGGGACAATG GTTTCGGGTC CACGTTAACT TTCTTTCGCC

                                                      BspEI
                                                          ~
 751  CCCGGCCCTG GTGAAACCGA CCCAAACCCT GACCCTGACC TGTACCTTTT
      GGGCCGGGAC CACTTTGGCT GGGTTTGGGA CTGGGACTGG ACATGGAAAA

      BspEI
      ~~~~~
 801  CCGGATTTAG CCTGTCCACG TCTGGCGTTG GCGTGGGCTG GATTCGCCAG
      GGCCTAAATC GGACAGGTGC AGACCGCAAC CGCACCCGAC CTAAGCGGTC

                 XhoI
                ~~~~~~~
                 AvaI
                ~~~~~~~
 851  CCGCCTGGGA AAGCCCTCGA GTGGCTGGCT CTGATTGATT GGGATGATGA
      GGCGGACCCT TTCGGGAGCT CACCGACCGA GACTAACTAA CCCTACTACT

 901  TAAGTATTAT AGCACCAGCC TGAAAACGCG TCTGACCATT AGCAAAGATA
      ATTCATAATA TCGTGGTCGG ACTTTTGCGC AGACTGGTAA TCGTTTCTAT

        BstBI
       ~~~~~~
        SfuI
       ~~~~~~
        NspV
       ~~~~~~
 951  CTTCGAAAAA TCAGGTGGTG CTGACTATGA CCAACATGGA CCCGGTGGAT
      GAAGCTTTTT AGTCCACCAC GACTGATACT GGTTGTACCT GGGCCACCTA

                      BssHII
                     ~~~~~~~
1001  ACGGCCACCT ATTATTGCGC GCGTTCTCCT CGTTATCGTG GTGCTTTTGA
      TGCCGGTGGA TAATAACGCG CGCAAGAGGA GCAATAGCAC CACGAAAACT

                                          BlpI
                                         ~~~~~~~
                 StyI                     CelII
               ~~~~~~~                   ~~~~~~~
1051  TTATTGGGGC CAAGGCACCC TGGTGACGGT TAGCTCAGCG TCGACCAAAG
      AATAACCCCG GTTCCGTGGG ACCACTGCCA ATCGAGTCGC AGCTGGTTTC

1101  GTCCAAGCGT GTTTCCGCTG GCTCCGAGCA GCAAAAGCAC CAGCGGCGGC
      CAGGTTCGCA CAAAGGCGAC CGAGGCTCGT CGTTTTCGTG GTCGCCGCCG

1151  ACGGCTGCCC TGGGCTGCCT GGTTAAAGAT TATTTCCCGG AACCAGTCAC
```

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       TGCCGACGGG ACCCGACGGA CCAATTTCTA ATAAAGGGCC TTGGTCAGTG

1201   CGTGAGCTGG AACAGCGGGG CGCTGACCAG CGGCGTGCAT ACCTTTCCGG
       GCACTCGACC TTGTCGCCCC GCGACTGGTC GCCGCACGTA TGGAAAGGCC

1251   CGGTGCTGCA AAGCAGCGGC CTGTATAGCC TGAGCAGCGT TGTGACCGTG
       GCCACGACGT TTCGTCGCCG GACATATCGG ACTCGTCGCA ACACTGGCAC

1301   CCGAGCAGCA GCTTAGGCAC TCAGACCTAT ATTTGCAACG TGAACCATAA
       GGCTCGTCGT CGAATCCGTG AGTCTGGATA TAAACGTTGC ACTTGGTATT

                                                    EcoRI
                                                   ~~~~~~
1351   ACCGAGCAAC ACCAAAGTGG ATAAAAAAGT GGAACCGAAA AGCGAATTCG
       TGGCTCGTTG TGGTTTCACC TATTTTTTCA CCTTGGCTTT TCGCTTAAGC

                                BssHII
                                ~~~~~~
1401   ACTATAAAGA TGACGATGAC AAAGGCGCGC CGTGGAGCCA CCCGCAGTTT
       TGATATTTCT ACTGCTACTG TTTCCGCGCG GCACCTCGGT GGGCGTCAAA

                  HindIII
                  ~~~~~~
1451   GAAAAATGAT AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG
       CTTTTTACTA TTCGAACTGG ACACTTCACT TTTTACCGCG TCTAACACGC
                             OGIII3  100.0%
                         ====================

1501   ACATTTTTTT TGTCTGCCGT TTAATTAAAG GGGGGGGGGG GCCGGCCTGG
       TGTAAAAAAA ACAGACGGCA AATTAATTTC CCCCCCCCCC CGGCCGGACC

1551   GGGGGGGTGT ACATGAAATT GTAAACGTTA ATATTTTGTT AAAATTCGCG
       CCCCCCCACA TGTACTTTAA CATTTGCAAT TATAAAACAA TTTTAAGCGC

1601   TTAAATTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG CCGAAATCGG
       AATTTAAAAA CAATTTAGTC GAGTAAAAAA TTGGTTATCC GGCTTTAGCC

1651   CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTTG
       GTTTTAGGGA ATATTTAGTT TTCTTATCTG GCTCTATCCC AACTCACAAC

1701   TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC
       AAGGTCAAAC CTTGTTCTCA GGTGATAATT TCTTGCACCT GAGGTTGCAG

1751   AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GGCCCACTAC GAGAACCATC
       TTTCCCGCTT TTTGGCAGAT AGTCCCGCTA CCGGGTGATG CTCTTGGTAG

1801   ACCCTAATCA AGTTTTTTGG GGTCGAGGTG CCGTAAAGCA CTAAATCGGA
       TGGGATTAGT TCAAAAAACC CCAGCTCCAC GGCATTTCGT GATTTAGCCT

1851   ACCCTAAAGG GAGCCCCCGA TTTAGAGCTT GACGGGGAAA GCCGGCGAAC
       TGGGATTTCC CTCGGGGGCT AAATCTCGAA CTGCCCCTTT CGGCCGCTTG

1901   GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG CTAGGGCGCT
       CACCGCTCTT TCCTTCCCTT CTTTCGCTTT CCTCGCCCGC GATCCCGCGA

1951   GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA
       CCGTTCACAT CGCCAGTGCG ACGCGCATTG GTGGTGTGGG CGGCGCGAAT
```

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2001   ATGCGCCGCT ACAGGGCGCG TGCTAGACTA GTGTTTAAAC CGGACCGGGG
       TACGCGGCGA TGTCCCGCGC ACGATCTGAT CACAAATTTG GCCTGGCCCC

2051   GGGGGCTTAA GTGGGCTGCA AAACAAAACG GCCTCCTGTC AGGAAGCCGC
       CCCCCGAATT CACCCGACGT TTTGTTTTGC CGGAGGACAG TCCTTCGGCG

2101   TTTTATCGGG TAGCCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG
       AAAATAGCCC ATCGGAGTGA CGGGCGAAAG GTCAGCCCTT TGGACAGCAC

2151   CCAGCTGCAT CAGTGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGCGTA
       GGTCGACGTA GTCACTTAGC CGGTTGCGCG CCCCTCTCCG CCAAACGCAT

2201   TTGGGAGCCA GGGTGGTTTT TCTTTTCACC AGTGAGACGG GCAACAGCTG
       AACCCTCGGT CCCACCAAAA AGAAAAGTGG TCACTCTGCC CGTTGTCGAC

2251   ATTGCCCTTC ACCGCCTGGC CCTGAGAGAG TTGCAGCAAG CGGTCCACGC
       TAACGGGAAG TGGCGGACCG GGACTCTCTC AACGTCGTTC GCCAGGTGCG

2301   TGGTTTGCCC CAGCAGGCGA AAATCCTGTT TGATGGTGGT CAGCGGCGGG
       ACCAAACGGG GTCGTCCGCT TTTAGGACAA ACTACCACCA GTCGCCGCCC

2351   ATATAACATG AGCTGTCCTC GGTATCGTCG TATCCCACTA CCGAGATGTC
       TATATTGTAC TCGACAGGAG CCATAGCAGC ATAGGGTGAT GGCTCTACAG

2401   CGCACCAACG CGCAGCCCGG ACTCGGTAAT GGCACGCATT GCGCCCAGCG
       GCGTGGTTGC GCGTCGGGCC TGAGCCATTA CCGTGCGTAA CGCGGGTCGC

2451   CCATCTGATC GTTGGCAACC AGCATCGCAG TGGGAACGAT GCCCTCATTC
       GGTAGACTAG CAACCGTTGG TCGTAGCGTC ACCCTTGCTA CGGGAGTAAG

2501   AGCATTTGCA TGGTTTGTTG AAAACCGGAC ATGGCACTCC AGTCGCCTTC
       TCGTAAACGT ACCAAACAAC TTTTGGCCTG TACCGTGAGG TCAGCGGAAG

2551   CCGTTCCGCT ATCGGCTGAA TTTGATTGCG AGTGAGATAT TTATGCCAGC
       GGCAAGGCGA TAGCCGACTT AAACTAACGC TCACTCTATA AATACGGTCG

2601   CAGCCAGACG CAGACGCGCC GAGACAGAAC TTAATGGGCC AGCTAACAGC
       GTCGGTCTGC GTCTGCGCGG CTCTGTCTTG AATTACCCGG TCGATTGTCG

2651   GCGATTTGCT GGTGGCCCAA TGCGACCAGA TGCTCCACGC CCAGTCGCGT
       CGCTAAACGA CCACCGGGTT ACGCTGGTCT ACGAGGTGCG GGTCAGCGCA

2701   ACCGTCCTCA TGGGAGAAAA TAATACTGTT GATGGGTGTC TGGTCAGAGA
       TGGCAGGAGT ACCCTCTTTT ATTATGACAA CTACCCACAG ACCAGTCTCT

2751   CATCAAGAAA TAACGCCGGA ACATTAGTGC AGGCAGCTTC CACAGCAATA
       GTAGTTCTTT ATTGCGGCCT TGTAATCACG TCCGTCGAAG GTGTCGTTAT

2801   GCATCCTGGT CATCCAGCGG ATAGTTAATA ATCAGCCCAC TGACACGTTG
       CGTAGGACCA GTAGGTCGCC TATCAATTAT TAGTCGGGTG ACTGTGCAAC

                     ApaLI
                    ~~~~~~
2851   CGCGAGAAGA TTGTGCACCG CCGCTTTACA GGCTTCGACG CCGCTTCGTT
       GCGCTCTTCT AACACGTGGC GGCGAAATGT CCGAAGCTGC GGCGAAGCAA
```

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2901  CTACCATCGA CACGACCACG CTGGCACCCA GTTGATCGGC GCGAGATTTA
      GATGGTAGCT GTGCTGGTGC GACCGTGGGT CAACTAGCCG CGCTCTAAAT

2951  ATCGCCGCGA CAATTTGCGA CGGCGCGTGC AGGGCCAGAC TGGAGGTGGC
      TAGCGGCGCT GTTAAACGCT GCCGCGCACG TCCCGGTCTG ACCTCCACCG

3001  AACGCCAATC AGCAACGACT GTTTGCCCGC CAGTTGTTGT GCCACGCGGT
      TTGCGGTTAG TCGTTGCTGA CAAACGGGCG GTCAACAACA CGGTGCGCCA

3051  TAGGAATGTA ATTCAGCTCC GCCATCGCCG CTTCCACTTT TTCCCGCGTT
      ATCCTTACAT TAAGTCGAGG CGGTAGCGGC GAAGGTGAAA AAGGGCGCAA

3101  TTCGCAGAAA CGTGGCTGGC CTGGTTCACC ACGCGGGAAA CGGTCTGATA
      AAGCGTCTTT GCACCGACCG GACCAAGTGG TGCGCCCTTT GCCAGACTAT

3151  AGAGACACCG GCATACTCTG CGACATCGTA TAACGTTACT GGTTTCACAT
      TCTCTGTGGC CGTATGAGAC GCTGTAGCAT ATTGCAATGA CCAAAGTGTA

3201  TCACCACCCT GAATTGACTC TCTTCCGGGC GCTATCATGC CATACCGCGA
      AGTGGTGGGA CTTAACTGAG AGAAGGCCCG CGATAGTACG GTATGGCGCT

3251  AAGGTTTTGC GCCATTCGAT GCTAGCCATG TGAGCAAAAG GCCAGCAAAA
      TTCCAAAACG CGGTAAGCTA CGATCGGTAC ACTCGTTTTC CGGTCGTTTT

3301  GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC
      CCGGTCCTTG GCATTTTTCC GGCGCAACGA CCGCAAAAAG GTATCCGAGG

3351  GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA
      CGGGGGGACT GCTCGTAGTG TTTTTAGCTG CGAGTTCAGT CTCCACCGCT

3401  AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT
      TTGGGCTGTC CTGATATTTC TATGGTCCGC AAAGGGGGAC CTTCGAGGGA

3451  CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT
      GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG GACAGGCGGA

3501  TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT
      AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA

                                                ApaLI
                                               ~~~~~~~
3551  CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC
      GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC ACGTGCTTGG

3601  CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT
      GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA

3651  CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC
      GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG GTGACCATTG

3701  AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG
      TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC

3751  GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC
      CACCGGATTG ATGCCGATGT GATCTTCTTG TCATAAACCA TAGACGCGAG

3801  TGCTGTAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC
```

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      ACGACATCGG TCAATGGAAG CCTTTTTCTC AACCATCGAG AACTAGGCCG

3851  AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT
      TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT TCGTCGTCTA

3901  TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG
      ATGCGCGTCT TTTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC

3951  GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCAGA
      CCAGACTGCG AGTCACCTTG CTTTTGAGTG CAATTCCCTA AAACCAGTCT

4001  TCTAGCACCA GGCGTTTAAG GGCACCAATA ACTGCCTTAA AAAAATTACG
      AGATCGTGGT CCGCAAATTC CCGTGGTTAT TGACGGAATT TTTTTAATGC

4051  CCCCGCCCTG CCACTCATCG CAGTACTGTT GTAATTCATT AAGCATTCTG
      GGGGCGGGAC GGTGAGTAGC GTCATGACAA CATTAAGTAA TTCGTAAGAC

4101  CCGACATGGA AGCCATCACA AACGGCATGA TGAACCTGAA TCGCCAGCGG
      GGCTGTACCT TCGGTAGTGT TTGCCGTACT ACTTGGACTT AGCGGTCGCC

4151  CATCAGCACC TTGTCGCCTT GCGTATAATA TTTGCCCATA GTGAAAACGG
      GTAGTCGTGG AACAGCGGAA CGCATATTAT AAACGGGTAT CACTTTTGCC

4201  GGGCGAAGAA GTTGTCCATA TTGGCTACGT TTAAATCAAA ACTGGTGAAA
      CCCGCTTCTT CAACAGGTAT AACCGATGCA AATTTAGTTT TGACCACTTT

4251  CTCACCCAGG GATTGGCTGA GACGAAAAAC ATATTCTCAA TAAACCCTTT
      GAGTGGGTCC CTAACCGACT CTGCTTTTTG TATAAGAGTT ATTTGGGAAA

4301  AGGGAAATAG GCCAGGTTTT CACCGTAACA CGCCACATCT TGCGAATATA
      TCCCTTTATC CGGTCCAAAA GTGGCATTGT GCGGTGTAGA ACGCTTATAT

4351  TGTGTAGAAA CTGCCGGAAA TCGTCGTGGT ATTCACTCCA GAGCGATGAA
      ACACATCTTT GACGGCCTTT AGCAGCACCA TAAGTGAGGT CTCGCTACTT

4401  AACGTTTCAG TTTGCTCATG GAAAACGGTG TAACAAGGGT GAACACTATC
      TTGCAAAGTC AAACGAGTAC CTTTTGCCAC ATTGTTCCCA CTTGTGATAG

4451  CCATATCACC AGCTCACCGT CTTTCATTGC CATACGGAAC TCCGGGTGAG
      GGTATAGTGG TCGAGTGGCA GAAAGTAACG GTATGCCTTG AGGCCCACTC

4501  CATTCATCAG GCGGGCAAGA ATGTGAATAA AGGCCGGATA AAACTTGTGC
      GTAAGTAGTC CGCCCGTTCT TACACTTATT TCCGGCCTAT TTTGAACACG

4551  TTATTTTTCT TTACGGTCTT TAAAAAGGCC GTAATATCCA GCTGAACGGT
      AATAAAAAGA AATGCCAGAA ATTTTTCCGG CATTATAGGT CGACTTGCCA

4601  CTGGTTATAG GTACATTGAG CAACTGACTG AAATGCCTCA AAATGTTCTT
      GACCAATATC CATGTAACTC GTTGACTGAC TTTACGGAGT TTTACAAGAA

4651  TACGATGCCA TTGGGATATA TCAACGGTGG TATATCCAGT GATTTTTTTC
      ATGCTACGGT AACCCTATAT AGTTGCCACC ATATAGGTCA CTAAAAAAAG

4701  TCCATTTTAG CTTCCTTAGC TCCTGAAAAT CTCGATAACT CAAAAAATAC
      AGGTAAAATC GAAGGAATCG AGGACTTTTA GAGCTATTGA GTTTTTTATG

4751  GCCCGGTAGT GATCTTATTT CATTATGGTG AAAGTTGGAA CCTCACCCGA
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        CGGGCCATCA CTAGAATAAA GTAATACCAC TTTCAACCTT GGAGTGGGCT

   4801 CGTCTAATGT GAGTTAGCTC ACTCATTAGG CACCCCAGGC TTTACACTTT
        GCAGATTACA CTCAATCGAG TGAGTAATCC GTGGGGTCCG AAATGTGAAA

   4851 ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA
        TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT

        M13 Reverse  primer 100.0%            XbaI
          ==================                ~~~~~~
   4901 CACAGGAAAC AGCTATGACC ATGATTACGA ATTTCTAGAT AACGAGGGCA
        GTGTCCTTTG TCGATACTGG TACTAATGCT TAAAGATCTA TTGCTCCCGT

   4951 AAAAATGAAA AAGACAGCTA TCGCGATTGC AGTGGCACTG GCTGGTTTCG
        TTTTTACTTT TTCTGTCGAT AGCGCTAACG TCACCGTGAC CGACCAAAGC

                      EcoRV
                        ~~~
   5001 CTACCGTAGC GCAGGCCGAT
        GATGGCATCG CGTCCGGCTA
```

Figure 14

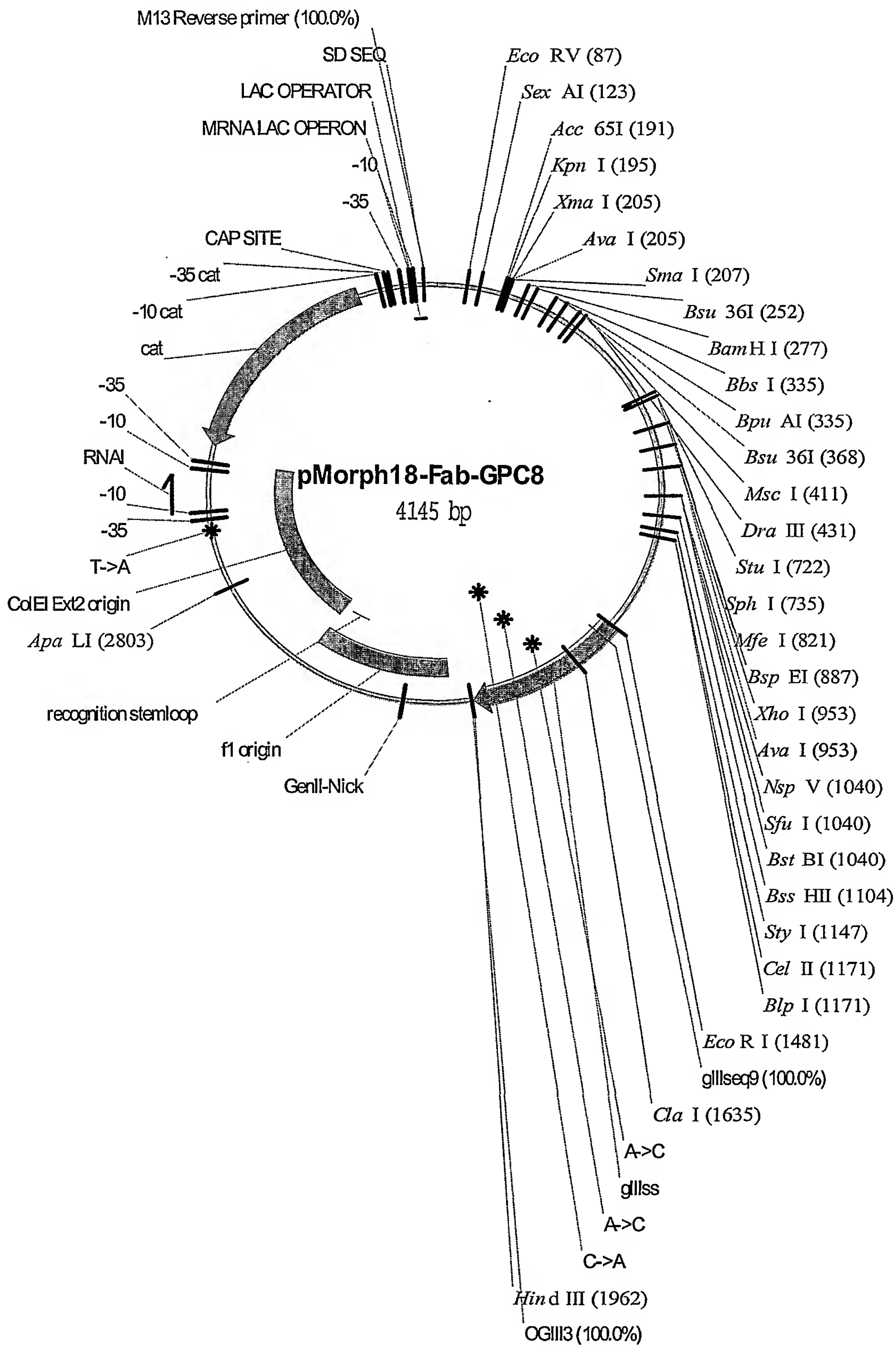
M13 Reverse primer (100.0%)
SD SEQ
LAC OPERATOR
MRNA LAC OPERON
-10
-35
CAP SITE
-35 cat
-10 cat
cat
-35
-10
RNAI
-10
-35
T->A
ColEI Ext2 origin
Apa LI (2803)
recognition stemloop
f1 origin
GenII-Nick
pMorph18-Fab-GPC8
4145 bp
Eco RV (87)
Sex AI (123)
Acc 65I (191)
Kpn I (195)
Xma I (205)
Ava I (205)
Sma I (207)
Bsu 36I (252)
Bam H I (277)
Bbs I (335)
Bpu AI (335)
Bsu 36I (368)
Msc I (411)
Dra III (431)
Stu I (722)
Sph I (735)
Mfe I (821)
Bsp EI (887)
Xho I (953)
Ava I (953)
Nsp V (1040)
Sfu I (1040)
Bst BI (1040)
Bss HII (1104)
Sty I (1147)
Cel II (1171)
Blp I (1171)
Eco R I (1481)
gIIIseq9 (100.0%)
Cla I (1635)
A->C
gIIIss
A->C
C->A
Hin d III (1962)
OGIII3 (100.0%)

Figure 14 (cont)

```
  1  TCAGATAACG AGGGCAAAAA ATGAAAAAGA CAGCTATCGC GATTGCAGTG
     AGTCTATTGC TCCCGTTTTT TACTTTTTCT GTCGATAGCG CTAACGTCAC

                                             EcoRV
                                            ~~~~~~
 51  GCACTGGCTG GTTTCGCTAC CGTAGCGCAG GCCGATATCG TGCTGACCCA
     CGTGACCGAC CAAAGCGATG GCATCGCGTC CGGCTATAGC ACGACTGGGT

                                SexAI
                               ~~~~~~~
101  GCCGCCTTCA GTGAGTGGCG CACCAGGTCA GCGTGTGACC ATCTCGTGTA
     CGGCGGAAGT CACTCACCGC GTGGTCCAGT CGCACACTGG TAGAGCACAT

                                                   KpnI
                                                  ~~~~~~~
                                                  Acc65I
                                                  ~~~~~~~
151  GCGGCAGCAG CAGCAACATT GGCAGCAACT ATGTGAGCTG GTACCAGCAG
     CGCCGTCGTC GTCGTTGTAA CCGTCGTTGA TACACTCGAC CATGGTCGTC

         XmaI
        ~~~~~~
         SmaI
        ~~~~~~
         AvaI                                        Bsu36I
        ~~~~~~                                            ~
201  TTGCCCGGGA CGGCGCCGAA ACTGCTGATT TATGATAACA ACCAGCGTCC
     AACGGGCCCT GCCGCGGCTT TGACGACTAA ATACTATTGT TGGTCGCAGG

     Bsu36I                          BamHI
     ~~~~~~                         ~~~~~~~
251  CTCAGGCGTG CCGGATCGTT TTAGCGGATC CAAAAGCGGC ACCAGCGCGA
     GAGTCCGCAC GGCCTAGCAA AATCGCCTAG GTTTTCGCCG TGGTCGCGCT

                                       BpuAI
                                      ~~~~~~~
                                       BbsI
                                      ~~~~~~~
301  GCCTTGCGAT TACGGGCCTG CAAAGCGAAG ACGAAGCGGA TTATTATTGC
     CGGAACGCTA ATGCCCGGAC GTTTCGCTTC TGCTTCGCCT AATAATAACG

                          Bsu36I
                          ~~~~~~~~
351  CAGAGCTATG ACATGCCTCA GGCTGTGTTT GGCGGCGGCA CGAAGTTTAA
     GTCTCGATAC TGTACGGAGT CCGACACAAA CCGCCGCCGT GCTTCAAATT

             MscI                  DraIII
            ~~~~~~~               ~~~~~~~~~~
401  CCGTTCTTGG CCAGCCGAAA GCCGCACCGA GTGTGACGCT GTTTCCGCCG
     GGCAAGAACC GGTCGGCTTT CGGCGTGGCT CACACTGCGA CAAAGGCGGC

451  AGCAGCGAAG AATTGCAGGC GAACAAAGCG ACCCTGGTGT GCCTGATTAG
     TCGTCGCTTC TTAACGTCCG CTTGTTTCGC TGGGACCACA CGGACTAATC

501  CGACTTTTAT CCGGGAGCCG TGACAGTGGC CTGGAAGGCA GATAGCAGCC
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       GCTGAAAATA GGCCCTCGGC ACTGTCACCG GACCTTCCGT CTATCGTCGG

 551   CCGTCAAGGC GGGAGTGGAG ACCACCACAC CCTCCAAACA AAGCAACAAC
       GGCAGTTCCG CCCTCACCTC TGGTGGTGTG GGAGGTTTGT TTCGTTGTTG

 601   AAGTACGCGG CCAGCAGCTA TCTGAGCCTG ACGCCTGAGC AGTGGAAGTC
       TTCATGCGCC GGTCGTCGAT AGACTCGGAC TGCGGACTCG TCACCTTCAG

 651   CCACAGAAGC TACAGCTGCC AGGTCACGCA TGAGGGGAGC ACCGTGGAAA
       GGTGTCTTCG ATGTCGACGG TCCAGTGCGT ACTCCCCTCG TGGCACCTTT

                              StuI         SphI
                             ~~~~~~~      ~~~~~~~
 701   AAACCGTTGC GCCGACTGAG GCCTGATAAG CATGCGTAGG AGAAAATAAA
       TTTGGCAACG CGGCTGACTC CGGACTATTC GTACGCATCC TCTTTTATTT

 751   ATGAAACAAA GCACTATTGC ACTGGCACTC TTACCGTTGC TCTTCACCCC
       TACTTTGTTT CGTGATAACG TGACCGTGAG AATGGCAACG AGAAGTGGGG

                                MfeI
                               ~~~~~~~
 801   TGTTACCAAA GCCCAGGTGC AATTGAAAGA AAGCGGCCCG GCCCTGGTGA
       ACAATGGTTT CGGGTCCACG TTAACTTTCT TTCGCCGGGC CGGGACCACT

                                                BspEI
                                               ~~~~~~~
 851   AACCGACCCA AACCCTGACC CTGACCTGTA CCTTTTCCGG ATTTAGCCTG
       TTGGCTGGGT TTGGGACTGG GACTGGACAT GGAAAAGGCC TAAATCGGAC

 901   TCCACGTCTG GCGTTGGCGT GGGCTGGATT CGCCAGCCGC CTGGGAAAGC
       AGGTGCAGAC CGCAACCGCA CCCGACCTAA GCGGTCGGCG GACCCTTTCG

        XhoI
       ~~~~~~
        AvaI
       ~~~~~~
 951   CCTCGAGTGG CTGGCTCTGA TTGATTGGGA TGATGATAAG TATTATAGCA
       GGAGCTCACC GACCGAGACT AACTAACCCT ACTACTATTC ATAATATCGT

                                                  BstBI
                                                 ~~~~~~~
                                                  SfuI
                                                 ~~~~~~~
                                                  NspV
                                                 ~~~~~~~
1001   CCAGCCTGAA AACGCGTCTG ACCATTAGCA AAGATACTTC GAAAAATCAG
       GGTCGGACTT TTGCGCAGAC TGGTAATCGT TTCTATGAAG CTTTTTAGTC

1051   GTGGTGCTGA CTATGACCAA CATGGACCCG GTGGATACGG CCACCTATTA
       CACCACGACT GATACTGGTT GTACCTGGGC CACCTATGCC GGTGGATAAT

        BssHII                                         StyI
       ~~~~~~                                         ~~~~~
1101   TTGCGCGCGT TCTCCTCGTT ATCGTGGTGC TTTTGATTAT TGGGGCCAAG
       AACGCGCGCA AGAGGAGCAA TAGCACCACG AAAACTAATA ACCCCGGTTC

                              BlpI
```

```
                         ~~~~~~~~
       StyI               CelII
       ~                 ~~~~~~~~
1151   GCACCCTGGT GACGGTTAGC TCAGCGTCGA CCAAAGGTCC AAGCGTGTTT
       CGTGGGACCA CTGCCAATCG AGTCGCAGCT GGTTTCCAGG TTCGCACAAA

1201   CCGCTGGCTC CGAGCAGCAA AAGCACCAGC GGCGGCACGG CTGCCCTGGG
       GGCGACCGAG GCTCGTCGTT TTCGTGGTCG CCGCCGTGCC GACGGGACCC

1251   CTGCCTGGTT AAAGATTATT TCCCGGAACC AGTCACCGTG AGCTGGAACA
       GACGGACCAA TTTCTAATAA AGGGCCTTGG TCAGTGGCAC TCGACCTTGT

1301   GCGGGGCGCT GACCAGCGGC GTGCATACCT TTCCGGCGGT GCTGCAAAGC
       CGCCCCGCGA CTGGTCGCCG CACGTATGGA AAGGCCGCCA CGACGTTTCG

1351   AGCGGCCTGT ATAGCCTGAG CAGCGTTGTG ACCGTGCCGA GCAGCAGCTT
       TCGCCGGACA TATCGGACTC GTCGCAACAC TGGCACGGCT CGTCGTCGAA

1401   AGGCACTCAG ACCTATATTT GCAACGTGAA CCATAAACCG AGCAACACCA
       TCCGTGAGTC TGGATATAAA CGTTGCACTT GGTATTTGGC TCGTTGTGGT

                                         EcoRI
                                        ~~~~~~~
1451   AAGTGGATAA AAAAGTGGAA CCGAAAAGCG AATTCGGGGG AGGGAGCGGG
       TTCACCTATT TTTTCACCTT GGCTTTTCGC TTAAGCCCCC TCCCTCGCCC

1501   AGCGGTGATT TTGATTATGA AAAGATGGCA AACGCTAATA AGGGGGCTAT
       TCGCCACTAA AACTAATACT TTTCTACCGT TTGCGATTAT TCCCCCGATA
                                    gIIIseq9  100.0%
                                 ====================

1551   GACCGAAAAT GCCGATGAAA ACGCGCTACA GTCTGACGCT AAAGGCAAAC
       CTGGCTTTTA CGGCTACTTT TGCGCGATGT CAGACTGCGA TTTCCGTTTG

                                           ClaI
                                          ~~~~~~
1601   TTGATTCTGT CGCTACTGAT TACGGTGCTG CTATCGATGG TTTCATTGGT
       AACTAAGACA GCGATGACTA ATGCCACGAC GATAGCTACC AAAGTAACCA

1651   GACGTTTCCG GCCTTGCTAA TGGTAATGGT GCTACTGGTG ATTTTGCTGG
       CTGCAAAGGC CGGAACGATT ACCATTACCA CGATGACCAC TAAAACGACC

1701   CTCTAATTCC CAAATGGCTC AAGTCGGTGA CGGTGATAAT TCACCTTTAA
       GAGATTAAGG GTTTACCGAG TTCAGCCACT GCCACTATTA AGTGGAAATT

1751   TGAATAATTT CCGTCAATAT TTACCTTCCC TCCCTCAATC GGTTGAATGT
       ACTTATTAAA GGCAGTTATA AATGGAAGGG AGGGAGTTAG CCAACTTACA

1801   CGCCCTTTTG TCTTTGGCGC TGGTAAACCA TATGAATTTT CTATTGATTG
       GCGGGAAAAC AGAAACCGCG ACCATTTGGT ATACTTAAAA GATAACTAAC

1851   TGACAAAATA AACTTATTCC GTGGTGTCTT TGCGTTTCTT TTATATGTTG
       ACTGTTTTAT TTGAATAAGG CACCACAGAA ACGCAAAGAA AATATACAAC

1901   CCACCTTTAT GTATGTATTT TCTACGTTTG CTAACATACT GCGTAATAAG
       GGTGGAAATA CATACATAAA AGATGCAAAC GATTGTATGA CGCATTATTC
```

```
                HindIII
                ~~~~~~
1951   GAGTCTTGAT AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG
       CTCAGAACTA TTCGAACTGG ACACTTCACT TTTTACCGCG TCTAACACGC
                               OGIII3  100.0%
                           ====================

2001   ACATTTTTTT TGTCTGCCGT TTAATGAAAT TGTAAACGTT AATATTTTGT
       TGTAAAAAAA ACAGACGGCA AATTACTTTA ACATTTGCAA TTATAAAACA

2051   TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT TAACCAATAG
       ATTTTAAGCG CAATTTAAAA ACAATTTAGT CGAGTAAAAA ATTGGTTATC

2101   GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG
       CGGCTTTAGC CGTTTTAGGG AATATTTAGT TTTCTTATCT GGCTCTATCC

2151   GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG
       CAACTCACAA CAAGGTCAAA CCTTGTTCTC AGGTGATAAT TTCTTGCACC

2201   ACTCCAACGT CAAAGGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA
       TGAGGTTGCA GTTTCCCGCT TTTTGGCAGA TAGTCCCGCT ACCGGGTGAT

2251   CGAGAACCAT CACCCTAATC AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC
       GCTCTTGGTA GTGGGATTAG TTCAAAAAAC CCCAGCTCCA CGGCATTTCG

2301   ACTAAATCGG AACCCTAAAG GGAGCCCCCG ATTTAGAGCT TGACGGGGAA
       TGATTTAGCC TTGGGATTTC CCTCGGGGGC TAAATCTCGA ACTGCCCCTT

2351   AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA AGGAGCGGGC
       TCGGCCGCTT GCACCGCTCT TTCCTTCCCT TCTTTCGCTT TCCTCGCCCG

2401   GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA CCACCACACC
       CGATCCCGCG ACCGTTCACA TCGCCAGTGC GACGCGCATT GGTGGTGTGG

2451   CGCCGCGCTT AATGCGCCGC TACAGGGCGC GTGCTAGCCA TGTGAGCAAA
       GCGGCGCGAA TTACGCGGCG ATGTCCCGCG CACGATCGGT ACACTCGTTT

2501   AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT
       TCCGGTCGTT TTCCGGTCCT TGGCATTTTT CCGGCGCAAC GACCGCAAAA

2551   TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT
       AGGTATCCGA GGCGGGGGGA CTGCTCGTAG TGTTTTTAGC TGCGAGTTCA

2601   CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC
       GTCTCCACCG CTTTGGGCTG TCCTGATATT TCTATGGTCC GCAAAGGGGG

2651   TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT
       ACCTTCGAGG GAGCACGCGA GAGGACAAGG CTGGGACGGC GAATGGCCTA

2701   ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCATAGCTCA
       TGGACAGGCG GAAAGAGGGA AGCCCTTCGC ACCGCGAAAG AGTATCGAGT

2751   CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG
       GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT TCGACCCGAC

        ApaLI
       ~~~~~~
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2801   TGTGCACGAA CCCCCCGTTC AGTCCGACCG CTGCGCCTTA TCCGGTAACT
       ACACGTGCTT GGGGGGCAAG TCAGGCTGGC GACGCGGAAT AGGCCATTGA

2851   ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
       TAGCAGAACT CAGGTTGGGC CATTCTGTGC TGAATAGCGG TGACCGTCGT

2901   GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA
       CGGTGACCAT TGTCCTAATC GTCTCGCTCC ATACATCCGC CACGATGTCT

2951   GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGA ACAGTATTTG
       CAAGAACTTC ACCACCGGAT TGATGCCGAT GTGATCTTCT TGTCATAAAC

3001   GTATCTGCGC TCTGCTGTAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC
       CATAGACGCG AGACGACATC GGTCAATGGA AGCCTTTTTC TCAACCATCG

3051   TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG
       AGAACTAGGC CGTTTGTTTG GTGGCGACCA TCGCCACCAA AAAAACAAAC

3101   CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA
       GTTCGTCGTC TAATGCGCGT CTTTTTTTCC TAGAGTTCTT CTAGGAAACT

3151   TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG
       AGAAAAGATG CCCCAGACTG CGAGTCACCT TGCTTTTGAG TGCAATTCCC

3201   ATTTTGGTCA GATCTAGCAC CAGGCGTTTA AGGGCACCAA TAACTGCCTT
       TAAAACCAGT CTAGATCGTG GTCCGCAAAT TCCCGTGGTT ATTGACGGAA

3251   AAAAAAATTA CGCCCCGCCC TGCCACTCAT CGCAGTACTG TTGTAATTCA
       TTTTTTTAAT GCGGGGCGGG ACGGTGAGTA GCGTCATGAC AACATTAAGT

3301   TTAAGCATTC TGCCGACATG GAAGCCATCA CAAACGGCAT GATGAACCTG
       AATTCGTAAG ACGGCTGTAC CTTCGGTAGT GTTTGCCGTA CTACTTGGAC

3351   AATCGCCAGC GGCATCAGCA CCTTGTCGCC TTGCGTATAA TATTTGCCCA
       TTAGCGGTCG CCGTAGTCGT GGAACAGCGG AACGCATATT ATAAACGGGT

3401   TAGTGAAAAC GGGGGCGAAG AAGTTGTCCA TATTGGCTAC GTTTAAATCA
       ATCACTTTTG CCCCCGCTTC TTCAACAGGT ATAACCGATG CAAATTTAGT

3451   AAACTGGTGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC
       TTTGACCACT TTGAGTGGGT CCCTAACCGA CTCTGCTTTT TGTATAAGAG

3501   AATAAACCCT TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT
       TTATTTGGGA AATCCCTTTA TCCGGTCCAA AAGTGGCATT GTGCGGTGTA

3551   CTTGCGAATA TATGTGTAGA AACTGCCGGA AATCGTCGTG GTATTCACTC
       GAACGCTTAT ATACACATCT TTGACGGCCT TTAGCAGCAC CATAAGTGAG

    +1
3601   CAGAGCGATG AAAACGTTTC AGTTTGCTCA TGGAAAACGG TGTAACAAGG
       GTCTCGCTAC TTTTGCAAAG TCAAACGAGT ACCTTTTGCC ACATTGTTCC

3651   GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT GCCATACGGA
       CACTTGTGAT AGGGTATAGT GGTCGAGTGG CAGAAAGTAA CGGTATGCCT

3701   ACTCCGGGTG AGCATTCATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA
       TGAGGCCCAC TCGTAAGTAG TCCGCCCGTT CTTACACTTA TTTCCGGCCT
```

```
3751   TAAAACTTGT GCTTATTTTT CTTTACGGTC TTTAAAAAGG CCGTAATATC
       ATTTTGAACA CGAATAAAAA GAAATGCCAG AAATTTTTCC GGCATTATAG

3801   CAGCTGAACG GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT
       GTCGACTTGC CAGACCAATA TCCATGTAAC TCGTTGACTG ACTTTACGGA

3851   CAAAATGTTC TTTACGATGC CATTGGGATA TATCAACGGT GGTATATCCA
       GTTTTACAAG AAATGCTACG GTAACCCTAT ATAGTTGCCA CCATATAGGT

3901   GTGATTTTTT TCTCCATTTT AGCTTCCTTA GCTCCTGAAA ATCTCGATAA
       CACTAAAAAA AGAGGTAAAA TCGAAGGAAT CGAGGACTTT TAGAGCTATT

3951   CTCAAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG TGAAAGTTGG
       GAGTTTTTTA TGCGGGCCAT CACTAGAATA AAGTAATACC ACTTTCAACC

4001   AACCTCACCC GACGTCTAAT GTGAGTTAGC TCACTCATTA GGCACCCCAG
       TTGGAGTGGG CTGCAGATTA CACTCAATCG AGTGAGTAAT CCGTGGGGTC

4051   GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG
       CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT AACACTCGCC

                  M13 Reverse  primer 100.0%
                     ===================
4101   ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATT
       TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAA
```

Figure 15

MS-GPC-1:

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARQYGHRGGFDHWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDFNE
SVFGGGTKLTVLG

MS-GPC-6

VH

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLE
WVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY
YCARGYGRYSPDLWGQGTLVTVSS

VL

DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLI
YGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYSNLPF
TFGQGTKVEIKRT

MS-GPC-8

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDMPQ
AVFGGGTKLTVLG

MS-GPC-10

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARQLHYRGGFDLWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDLTM
GVFGGGTKLTVLG

MS-GPC-8-6

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDYDH
YVFGGGTKLTVLG

MS-GPC-8-10

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS
VL
DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDLIRH
VFGGGTKLTVLG

MS-GPC-8-17
VH
QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS
VL
DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDFSV
YVFGGGTKLTVLG

MS-GPC-8-27
VH
QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS
VL
DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNYVSWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDMNV
HVFGGGTKLTVLG

MS-GPC-8-6-13

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSESNIGANYVTWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDYDH
YVFGGGTKLTVLG

MS-GPC-8-10-57

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSESNIGNNYVQWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDLIRH
VFGGGTKLTVLG

MS-GPC-8-27-41

VH

QVQLKESGPALVKPTQTLTLTCTFSGFSLSTSGVGVGWIRQPPGKALE
WLALIDWDDDKYYSTSLKTRLTISKDTSKNQVVLTMTNMDPVDTATYY
CARSPRYRGAFDYWGQGTLVTVSS

VL

DIVLTQPPSVSGAPGQRVTISCSGSESNIGNNYVQWYQQLPGTAPKLLI
YDNNQRPSGVPDRFSGSKSGTSASLAITGLQSEDEADYYCQSYDMNV
HVFGGGTKLTVLG

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/15625

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 39/395, 44
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 133.1, 138.1, 141.1, 143.1, 144.1, 152.1, 153.1, 155.1, 172.1, 173.1, 174.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, CANCERLIT, WEST
search terms antibodies, apoptosis, HLA-DR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	DUEYMES et al. Anti-endothelial cell antibody binding causes apoptosis of endothelial cells. Arthritis & Rheumatism. September 1997, Vol.40, page S103. See abstract.	1 --- 109, 110
X --- Y	KIM et al. Altered expression of the genes regulating apoptosis in multidrug resistant human myeloid leukemia cell lines overexpressing MDR1 or MRP gene. 1997, Vol.11, pages 945-950. See abstract.	1 --- 109,110
X --- Y	ISHIZUKA et al. Antitumour activity of murine monoclonal antibody NCC-ST-421 on human cancer cells by inducing apoptosis. Anticancer Research. July-August 1998, Vol.18, pages 2513-2518. See absract.	1 --- 109-110

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
29 JULY 2001

Date of mailing of the international search report
15 AUG 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
KAREN A CANELLA
Telephone No. (703) 308-1235

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	GHAHREMANI et al. Activation of fas ligand/receptor system kills ovarian cancer cell lines by apoptotic mechanism. Gynecological Oncology. August 1998, Vol.70, pages 275-281. See abstract.	1 --- 109, 110
X --- Y	HENSEL et al. Characterization of glycosylphosphatidylinositol-linked molecule CD55/decay-accelerating factor as the receptor for antibody SC-1-induced apoptosis. Cancer Research. 15 October 1999, Vol.59, pages 5299-5306. See abstract.	1 --- 109,110
X --- Y	NAKAMURA et al. Apoptosis induction of the human lung cancer cell line in multicellular heterospheroids with human antigangliosides GM2 monoclonal antibody. Cancer Research. 15 October 1999, Vol.59, pages 5323-5330. See abstract.	1 --- 109, 110
X --- Y	WALLEN-OHMAN et al. Antibody-induced apoptosis in a human leukemia cell line is energy dependent. Cancer Letters. 10 December 1993, Vol.75, pages 103-109. See abstract.	1 --- 109, 110
X --- Y	MYSLER et al. The apoptosis-1/Fas protein in human systmic lupus erythematosus. Journal of Clinical Investigation. March 1994, Vol.93, pages 1029-1034. See abstract.	1 --- 109, 110
X --- Y	ACKERMAN et al. Induction of apoptotic or lytic cell death in an ovarain adenocarcinoma cell line by antibodies generated against a synthetic N-terminal extracellular domain gonadotropin-releasing hormone receptor peptide. Cancer Letters. 30 June 1994, Vol.81, pages 177-184. See abstract.	1 --- 109, 110
X --- Y	ERAY et al. Cross-linking of surface IgG induces apoptosis in bcl-2 expressing human follicular lymphoma line of mature B cell phenotype. International Immunology. December 1994, Vol.6, pages 1817-1827. See abstract.	1 --- 109, 110
X --- Y	NAKAMURA et al. Apoptosis in human hepatoma cell line induced by 4,5-didehydro geranylgeranoic acid via down-regulation of transforming growth factor-alpha. Biochemical and Biophysical Research Communications. 06 February 1996, Vol.219, pages 100-104. See abstract.	1 --- 109, 110
X --- Y	VOLLMERS et al. Apoptosis of stomach carcinoma cells induced by a human monoclonal antibody. Cancer. 15 August 1995, Vol.76, pages 550-558. See abstract.	1 --- 109, 110
X --- Y	HATA et al. Fas/Apo-1 (CD95)-mediated and CD95-independent apoptosis of malignant plasma cells. Leukemia and Lymphoma. December 1996, Vol.24, pages 35-42. See abstract.	1 --- 109, 110

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HIGASHIGAWA et al. FK506 inhibits anti-IgM antibody-induced apoptosis and 17 kD endonuclease activity in the human B-cell line, MBC-1, established from Burkitt's lymphoma. British Journal of Haematology, December 1997, Vol.99, pages 908-913. See abstract.	1 --- 109, 110
X --- Y	MASUDA et al. Dual action of CD30 antigen: anti-CD30 antibody induced apoptosis and interleukin-8 secretion in Ki-1 lymphoma cells. International Journal of Hematology, April 1998, Vol.67, pages 257-265. See abstract.	1 --- 109, 110
X --- Y	HAYAKAWA et al. A short peptide derived from the antisense homology box of Fas ligand induces apoptosis in anti-Fas antibody-insensitive huamn ovarain cancer cells. Apoptosis. February 2000, Vol.5, pages 37-41. See entire document.	1 --- 109, 110
X --- Y	VIDOVIC et al. Selective apoptosis of neoplastic cells by the HLA-DR-specific monoclonal antibody. Cancer Letters. 19 June 1998, Vol.128, pages 127-135. See entire document.	1-5, 7-10, 18, 19, 21 ---------------- 6, 14, 15, 38, 66, 68, 69, 70, 109-114
X --- Y	LEE et al. HLA-DR-Triggered Inhibiton of Hemopoiesis Involves Fas/Fas Ligand Interactions and Is Prevented by c-kit Ligand. Journal of Immunology. 01 October 1997, Vol.159, pages 3211-3219. See entire document.	1-5, 7-10 ---------------- 6, 14, 15, 38, 69 109-114
X --- Y	LEE et al. HLA-DR-Mediated Signals for Hematopoiesis and Induction of Apoptosis Involve But Are Not Limited to a Nitric Oxide Pathway. Blood. 01 July 1997, Vol.90, pages 217-225. See entire document.	1-5, 7-10 ---------------- 6, 14, 15, 38, 69 109-114
X --- Y	McDEVITT et al. Monoclonal anti-Ia antibody therapy in animal models of autoimmune disease. Ciba Foundation Symposium. 1987, Vol.129, pages 184-193. See entire document.	1-5, 7-10, 66, 68, 70 ---------------- 6, 14, 15, 38, 69
Y	HARRISON et al. Screening of Phage Antibody Libraries. Methods in Enzymology. 1996, Vol.267, pages 83-109. See entire document.	6, 69
Y	ROOS et al. Establishment and characterization of a human EBV-negative B cell line. Leukemia Research. 1982, Vol.6, pages 685-693. See abstract.	14, 15

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TOSI et al. Immunochemical definition of the new dr specificity 8WDRw13. Immunological Communications. 1981, Vol.10, pages 275-292. See abstract.	14, 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/15625

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 133.1, 138.1, 141.1, 143.1, 144.1, 152.1, 153.1, 155.1, 172.1, 173.1, 174.1

INTERNATIONAL SEARCH REPORT

Inter ial application No.
PCT/US01/15625

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 11-13,24-37,39-65,71-108
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.